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# MEDICAL SCIENCES

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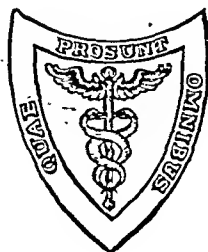
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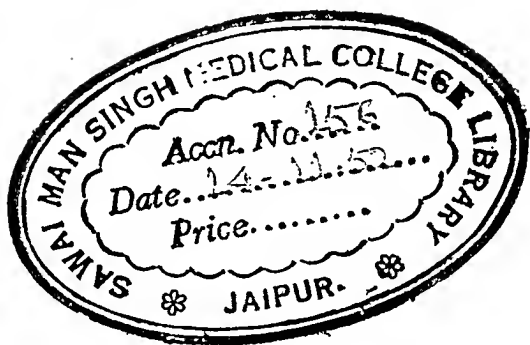
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# THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

JANUARY, 1944

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## ORIGINAL ARTICLES

### THE NATURE OF GRAVES' DISEASE WITH SPECIAL REFERENCE TO ITS OPHTHALMIC COMPONENT\*

BY J. H. MEANS, M.D.

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(From the Thyroid Clinic of the Massachusetts General Hospital)

AROUND the turn of the 18th century there practiced in that charming little English city of Bath, made vivid for us by Booth Tarkington in his "Monsieur Beaucaire," a popular, fashionable but likewise astute physician, one Caleb Hillier Parry.

Like most of the great clinicians of the 18th and 19th centuries, Parry was not only a keen observer, but in addition an eloquent recorder of what he observed. On one occasion, for example, he wrote, "There is one malady which I have in five cases seen coincident with what appeared to be enlargement of the heart, and which, so far as I know, has not been noticed, in that connection, by medical writers. The malady to which I allude is enlargement of the thyroid gland." In further description of one of these, Parry states, "The eyes were protruded from their sockets, and the countenance exhibited an appearance of agitation and distress especially on any muscular exertion, which I have rarely seen equalled." Of the heart action he notes, "Each systole . . . shook the whole trunk of the body."

Parry's<sup>47</sup> is the first adequate description of this disease, and it should by rights be called after him. Posterity has ordained, however, that the honor should be bestowed on others. In the English-speaking countries it falls to Robert Graves,<sup>19</sup> of Dublin, and in Germany, to von Basedow,<sup>7</sup> who was the last of the three to describe it. However, a more complete description of the eye phenomena was given by von Basedow than by either of the others.

You may well ask, Why make use of an eponym at all? My reply is that an etiologic label is still impossible because the etiology is unknown, and furthermore because I know of no descriptive term broad enough to fit all the cases which I believe come within the clinical entity. "Exophthalmic goiter" is not adequate because there

\* A lecture delivered before the Alpha of Tennessee Chapter of Alpha Omega Alpha, Vanderbilt University, January 29, 1943.



is not always exophthalmos. There is not always goiter. "Toxic goiter" will not do for they are not always toxic. Graves' disease it still has to be. Within the group so termed, I shall include those cases of thyrotoxicosis with diffuse hyperplasia of the thyroid, with or without the type of ophthalmic involvement described by Parry, Graves and Basedow, and those cases with the characteristic ophthalmic involvement whether or not they be accompanied by thyrotoxicosis.

In other words, whereas the classic picture of Graves' disease includes both toxic goiter and certain peculiar ophthalmic manifestations, there are also certain cases belonging, I am sure, within the clinical entity, in which there is characteristic ophthalmic involvement with but little or no accompanying thyrotoxicosis. There may actually be hypothyroidism. My colleagues and I,<sup>42,43</sup> of late, have adopted the practice of subdividing our cases of Graves' disease into the classic type and the special ophthalmopathic type.\* The latter includes what have variously been called in the literature "malignant exophthalmos,"<sup>34</sup> "exophthalmic ophthalmoplegia,"<sup>8,12</sup> "progressive postoperative exophthalmos with low basal metabolic rate,"<sup>18</sup> and so forth.<sup>29,64,65,66,70</sup> In the classic type, thyrotoxicosis and eye involvement are both present, and as the disease progresses the intensities of the two vary in parallel. Furthermore, in the classic type, the thyrotoxicosis is, from the therapeutic point of view, the major consideration. If it is relieved, the eye condition usually improves also without special treatment.

In the special ophthalmopathic type, on the other hand, the ophthalmopathy in its progression seems divorced or dissociated from the thyrotoxicosis; and from the therapeutic point of view the eyes, not the thyrotoxicosis, provide the chief indication for treatment.

I do not wish to imply that a sharp line can be drawn between the classic and special ophthalmopathic types of Graves' disease. Indeed, in a single case the picture may proceed from one type to the other, and in another case perhaps the subdivision cannot be made at all. However, there are cases, or stages in cases, where the subdivision can be made with ease, and I shall try to establish that it is of both physiologic interest and therapeutic importance to make it.

**Etiology.** Let us consider now for a few moments the general nature of Graves' disease. Is it infectious, neoplastic or a deficiency syndrome? Presumably none of these. What then? About all that can be said is that it is a breakdown of that balance of hormonal action which is necessary to the maintenance of health. We used to think of it in terms of disturbed thyroid function alone. Since the discovery of the thyroid stimulating hormone of the pituitary (TSH) it has been

\* An ophthalmologic colleague has objected to my use of "ophthalmopathie" on the ground that such an adjective refers to the eyeball rather than to the orbit. I find, however, that *ὀφθαλμός* means eye, and that eye means the organ of vision. Since we have a separate word eyeball, eye must be more inclusive, and I regard it as proper to use the word as including the eyeball's adnexa as well as the globe itself. Also I am impressed by the fact that ophthalmologists in caring for patients include the adnexa within their field. Furthermore, it is to be noted that for years the term "eye signs" has been used with reference to all the ocular manifestations of Graves' disease. If eye, then why not ophthalmie? and if ophthalmie, why not ophthalmopathie?

evident that the pituitary, at least, among the other endocrines is also concerned in the morbid physiology of Graves' disease. But this is not all, for it has been shown that there are changes in the thymus, the musculature, and in the lymphatic and reticulo-endothelial systems as well. Altogether it is a complicated derangement.

What causes the breakdown? Why do some persons suffer from it and others not? It has been thought that shocking experiences, stresses and strains of living in one way or another pull the trigger and cause the explosion which is the disease. Certainly it is true that one can frequently get histories in which what may be called psychic traumata are rapidly followed by the development of the disease. So too, as Hertz and Means<sup>22</sup> have shown, metabolic insults such as rapid and great weight loss, intentional or otherwise, may act in similar fashion. However, in many other cases no such history can be elicited and the onset is gradual and insidious instead of explosive. Furthermore many persons, most in fact, sustaining such impacts do not develop Graves' disease. Instead they may develop peptic ulcer, hypertension, ulcerative colitis or a psychosis.

This makes one think that in addition to a precipitating factor it is necessary, for the production of Graves' disease, also to have a predisposed individual. How predisposed? By heredity? Perhaps. Certainly it is not uncommon to find families with a high incidence of Graves' disease running through several generations. But these are greatly outnumbered by those cases in which no family story can be obtained. My own guess is that Graves' disease represents a breakdown under stress or strain in a given individual's weakest part. Only a few of us are like Oliver Wendell Holmes' "One Horse Shay" in which every part was made just as strong as every other part so that no part could give out before another. The result, as you should recall, was that the whole affair suddenly completely disintegrated after 100 years of use. This is analogous to the nonagenarian who passes away peacefully without any evidence of disease.

Most of us are not built that way. According to the nature of our constitutions one of us may fail to adjust to his environment in his endocrine system and develop Graves' disease, another in his gastrointestinal tract and develop an ulcer, yet a third in his circulatory system and develop irritable heart or effort syndrome.

Very recently I saw identical twin girls of 10. Cynthia had developed definite, but mild, classic Graves' disease. Carol was apparently quite normal. Why, with identical constitutions and environments, had they not either both developed the disease, or neither. The only clue I could get was in the mother's statement that Cynthia had been infested with and treated for roundworms of some sort just prior to the development of her Graves' disease, while Carol had escaped. I am rather expecting, however, to hear that Carol has developed the disease also. In passing, it is interesting to note that Cynthia, in the few months she had been ill, had gained an inch and a half in height over her sister. This growth acceleration in thyrotoxic children was pointed out by Hertz and Galli-Mainini<sup>21</sup> in 1941.

The onset and the course of Graves' disease are most variable. Time will not permit even an enumeration of the various patterns. I shall merely remind you that it may start with either thyrotoxic manifestations or ophthalmic, or with the two simultaneously. The onset may be abrupt or gradual. There may be one or more spontaneous remissions and relapses. There is a definite tendency for the disease to be self-limited. It tends, as we say, to burn itself out. The total duration may be only months, or it may be, if untreated, many years. It may occur in either sex and at any age. Sometimes it goes into a low-grade, chronic form.



FIG. 1.—Graves' disease in one of twins. (Kindness of Dr. Nathan B. Talbot.)

**Morbid Physiology.** Of the morbid physiology of Graves' disease more is known than of its etiology.

The thyroid gland is certainly under abnormal stimulation. In many instances, it delivers to the blood stream an excess of its hormone. Hyperthyroidism results. This is manifested clinically by the familiar symptoms of thyrotoxicosis, and in the laboratory by increased basal metabolic rate and elevation of the protein-bound iodine in the blood. Histologically it is manifested by high columnar epithelium.

The nature of the stimulus to the thyroid is not clear. For years it was thought to be nervous. But now, I think, the weight of evidence is against any direct nervous stimulation of thyroid cells to secrete. It is clear, of course, that the anterior pituitary makes a thyroid stimulating principle (TSH) and that the administration of this material will cause hyperthyroidism in animals and man, indeed, a good imitation of Graves' disease, because exophthalmos may be produced as well. There is also good evidence that the thyroid hormone suppresses the action of the pituitary with respect to the production of TSH,<sup>15,58,69</sup> that lack of it stimulates it<sup>56</sup> and thus emerges a balance between the two glands to which Salter<sup>59</sup> has given the term "pituitary-thyroid axis." Furthermore Galli-Mainini<sup>16</sup> has made observations, *in vitro* with the Warburg apparatus, which indicate that the thyroid hormone inhibits the thyroid cells also. That is to

say, as the concentration of thyroid hormone rises in the blood, a depressing action will be exerted on the thyroid cell. This work is yet to be confirmed.

The rôle of the pituitary in clinical Graves' disease is far from established. There has been some tendency to regard the disease as primary hyperpituitarism with secondary hyperthyroidism. That this is an accurate conception, I think doubtful. There is no evidence that the anterior lobe is commonly hyperplastic in Graves' disease and only inconclusive evidence that there is an excess of TSH in the blood. However, even so, the gland may be putting out an increased quantity because the blood level of TSH would not disclose its rate of manufacture, but merely the relation between rate of entry and departure from the blood stream.

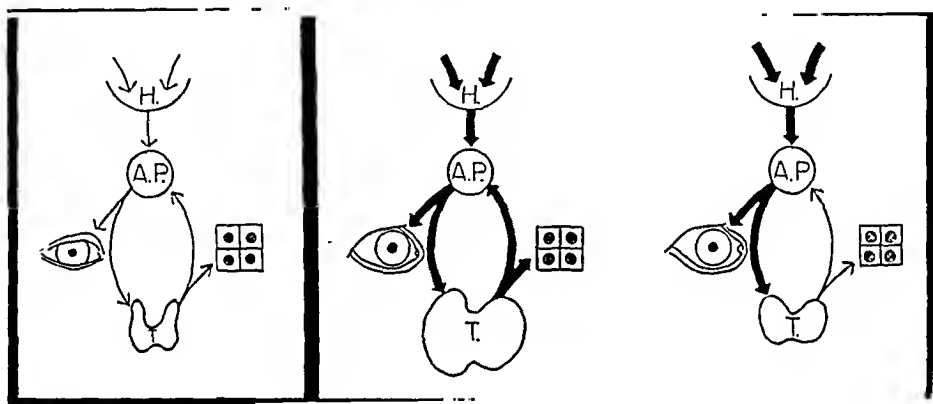


FIG. 2.—The pituitary-thyroid axis. In the *left-hand diagram*, as shown by arrows, a normally stimulated hypothalamus, *H.*, stimulates, in normal degree, the anterior pituitary, *A.P.*, to secrete its hormone which stimulates the thyroid gland, *T.*, to make its hormone which stimulates all tissue cells and causes an elevation in their metabolic rate. As further shown by arrows, the thyroid hormone also inhibits the *A.P.* and the thyrotropic hormone of the *A.P.* is believed to play some sort of direct rôle in the determination of the volume of orbital contents. In the *central diagram*, as shown by the heavier arrows, everything is intensified all around. The thyroid enlarges and hyperfunctionates, the ophthalmopathy develops. This is illustrative of what is believed to occur in classic Graves' disease. In the *right-hand diagram* we have the same, until the thyroid is reached. This gland, for reasons unknown, fails to respond to pituitary stimulation, at least to the same degree, hyperthyroidism is not produced, but the ophthalmopathy develops and progresses perhaps because sufficient inhibition of *A.P.* by *T.* is lacking. This is the state of affairs believed to exist in the ophthalmopathic type of Graves' disease.

If the mode of action of TSH on the thyroid cell be learned, light may be thrown on the nature of Graves' disease. Rawson<sup>57</sup> and his collaborators have made some important observations in this connection. In the first place, they found that if TSH be exposed as part of a substrate to thyroid cells growing in tissue culture, its physiologic activity is lost. This loss, however, they were able to show further, is the result of inactivation, not of degradation, of the hormone, because if treated with mild reducing agents such as glucose or vitamin C at the right temperature and pH, the physiologic activity could be restored. The hormone, under such circumstances is reactivated.

Physiologic activity is assayed by injecting the test material into day-old chicks and measuring the cell height of their thyroid epithelium. It looks, in other words, as though TSH affects the thyroid cell by acting as a catalyst on its oxidative enzyme system.

Rawson<sup>54</sup> also, by his tissue culture method, has observed the TSH-inactivating properties of various types of goiters removed surgically from patients. He finds that the thyroid tissue of thyrotoxic patients inactivates nearly all of the TSH added to the substrate, whereas equal amounts of normal thyroid tissue, obtained while operating on persons with parathyroid tumors, inactivates only one-half as much as that inactivated by the glands of thyrotoxic persons. In the case of non-toxic nodular goiters, there is no inactivation of TSH at all.

Some years ago, Hertz and Oastler<sup>23</sup> studied the thyrotropic activity of urine. In certain cases of myxedema they found a considerable amount, in normal persons only a slight amount, and in thyrotoxic patients none at all.

Rawson<sup>53</sup> has made similar observations, but in addition has studied both heated and unheated urine. The effect of heating is reduction, because urine contains various reducing agents, such as vitamin C and glucose.

In unheated urines the thyrotoxic persons, Rawson, like Hertz and Oastler, found no thyrotropic activity. After heating, however, a considerable thyrotropic activity developed. In the urine of normal persons, slight thyrotropic activity was found in the unheated, somewhat more in the heated, but less than in the cases of thyrotoxicosis. In persons with low metabolic rates, the unheated urine showed the most thyrotropic activity of all. In other words, the thyroid gland of persons with thyrotoxicosis has an increased power to inactivate TSH, and the greater total amount found in such cases suggests that there may be a greater production of TSH by the anterior pituitary.

In 2 cases of the special ophthalmopathic type of Graves' disease, Rawson found high TSH activity in the unheated urine. He concluded that in these, as in the thyrotoxic type, the anterior pituitary overacts with regard to TSH secretion, but for some reason unknown, the thyroids in these cases cannot respond to it, and TSH in consequence gets by, not inactivated, to be excreted in the urine.

Another approach to thyroid physiology has been provided by Hertz and his co-workers.<sup>24-28</sup> This depends upon the use of artificially radioactive iodine, which can be used in a tracer method of studying the function of the thyroid gland. The thyroid hormone contains iodine, and it is an ingredient essential to physiologic activity; therefore, thyroid cells in order to produce the hormone must have a supply of iodine. Lacking this, be the tissue ever so hyperplastic, no physiologically active thyroid hormone can be produced. Cyanates, for example, have been found to interfere, in some way not yet completely understood, with the elaboration of the thyroid hormone, and we have obtained a biopsy in the case of a man who developed a goiter while receiving potassium sulfocyanate as treatment for hypertension. The thyroid was wildly hyperplastic, but the patient was hypothyroid!<sup>55</sup>

By means of the radioactive (labeled) iodine, it has been found that the hyperplastic thyroid collects administered iodine at a much faster rate than does the normal, and this holds whether the increased effort of the thyroid cells to make hormone is the result of stimulation by TSH or through some other agency, such as a stoppage or bottleneck in the assembly line of hormone production, which is what we conceive happens in the case of cyanates and some similar substances.

Of thyroid physiology in Graves' disease at least it can be said that the cells of the thyroid are hyperfunctioning, or trying to hyperfunction, and that a factor in this functional alteration is an increased ability to inactivate TSH. It is to be presumed that the anterior pituitary, even though not visibly hyperplastic, makes an increased amount of TSH, because an increased amount, as compared with the normal, is found in the inactivated form in the urine.

Thus in Graves' disease we have evidence of an increased flow of the hormone which stimulates the thyroid, an increased avidity of the thyroid for iodine, a necessary ingredient of its hormone, and an increased flow of thyroid hormone which inhibits the pituitary. The pituitary-thyroid axis is geared at a higher level. Where the factor that causes this shift impinges on the axis, is not clear. Perhaps it is in the form of a nervous influence routed through the hypothalamus to the anterior lobe. Also other endocrines may play some minor rôle.

**Ophthalmic Component.** Let us now focus on the ophthalmic component of Graves' disease, indulge, in fact, in a little of what might be called endocrinologic ophthalmology.

In the 19th century there were many and excellent descriptions of the disease in general and of the eye signs in particular. Parry had noted the wild, staring appearance and the protrusion of the eyeballs. Von Basedow had linked proptosis or exophthalmos, violent heart action and goiter as the essential framework of the syndrome. After his home town, it came to be known in Europe as the Merseberg triad. The names of various ophthalmologists and others became attached to various special manifestations that accompanied the protrusion: Dalrymple's sign being retraction of the upper lid; von Graefe's, lag of the lid behind the globe in downward rotation; Stellwag's, widening of the palpebral slits and infrequent wrinkling; Moebius' sign, difficulty in converging the eyeballs in attempting to fix near objects; Jellinek's sign, pigmentation of the eyelids; Kocher's sign, in upward rotation, movement of the lid ahead of the eyeball (this sign is practically von Graefe's in reverse, perhaps we may be permitted to call it globe lag); and Joffroy's sign, absence of brow wrinkling on upward gaze with head lowered.

To this galaxy of 19th century eponymously labeled manifestations we may add yet others, which more recently have seemed important, such as edema of the eyelids, chemosis or edema of the global conjunctiva, ulceration of the cornea, epiphora, and visual defects such as diplopia, diminution in acuity of vision and (found by us in 1 case only) bitemporal hemianopsia.

I should like to make it clear that, in case-taking, there is no virtue

in merely recognizing and recording individually certain sonorously named eye signs. Indeed, I think there is but little good reason for burdening the memory with which man's name goes with which phenomenon. The real objective should be to size up the ophthalmic situation *in toto* and interpret its significance. What can one conclude, on the basis of observed phenomena, about what is actually going on in the patient? Is the lesion advancing, regressing or remaining stationary? Those are the things that must be known if treatment is to be planned intelligently.

Special attention to isolated eye signs is only indicated if, and when, they have some special significance. The last category of signs that I mentioned, edema of lids and conjunctiva, does, as will appear later, have a very special significance. There is a possibility that lid retraction, lid lag and globe lag (these are really but different aspects of a single abnormality, namely spasm of the levator palpebrae superioris



FIG. 3.—Graves' disease of special ophthalmopathie type in a man of 55 years. BMR —5 on iodine. No thyroidectomy. Extreme lid retraction, moderate proptosis O.U. Slight lid edema, no chemosis. (U. 301702.)

muscle) also have a special significance. All the other eye signs may be said to be related to, and determined by, the state of affairs within the orbit. The ophthalmic component of Graves' disease is an affliction of extraglobal orbital contents, not primarily of the eyeball. The eyeball is pushed forward and its membranes engorged because of what is going on behind it in the orbit, or the muscles controlling it, and the eyelids, or both, are the seat of pathologic alteration.

The first point to settle in the person suspected of having Graves' disease is whether eye signs, any eye signs, are present or absent. In early Graves' disease the only detectable abnormality may be staring expression of the eyes and lid lag. There may be no protrusion of the globes at all. We may, indeed, measure their position with an exophthalmometer and find it quite within the limits of normal. Despite the lack of exophthalmos, however, the staring, wild look is visible across the room and is close to pathognomonic as far as diagnostic import goes. It is due to the widening of the palpebral fissure,

Dalrymple's sign, with exposure of the white of the eye above the iris. A similar appearance might be found in states of real terror, or it can be produced voluntarily, that is to say, faked. When it is not faked nor the result of terror, then the sign is very strong evidence for Graves' disease. Often it is asymmetric or unilateral, as has been emphasized lately by Pochin.<sup>50,52</sup>

Years ago von Graefe pointed out that lid lag is independent of exophthalmos.\* He believed it to be of sympathetic origin due to an affection of the sympathetic innervation of the levator palpebræ superioris. Pochin,<sup>51</sup> however, maintains that while the lower lid is lowered by sympathetic stimulation, it is raised relative to the cornea when the upper lid is retracted by the levator palpebræ superioris. This is a striated muscle and is innervated, not by the sympathetic, but by the oculomotor nerve. The sympathetic overactivity theory of lid retraction, therefore, according to Pochin, is untenable. Indeed, I have concluded after searching the literature that the sympathetic has little if anything to do with any of the ophthalmic manifestations of Graves' disease. In certain types of experimental exophthalmos in animals, yes; in human Graves' disease, no. Nor do I believe that sympathectomy offers anything in the treatment of human exophthalmos, Jonnesco<sup>51</sup> to the contrary, notwithstanding.

At the onset of Graves' disease, thyrotoxic manifestations may be the first to appear, or the ophthalmic, or the two may appear together. Relationships of this character were noted as early as 1860 by Houlhouse (in discussion of C. H. Jones<sup>30</sup>). As I have already indicated, thyrotoxic and ophthalmic components may run parallel courses, or vary inversely or independently. These variations serve as a basis for the separation of cases into types.<sup>42,43</sup> Furthermore, either thyrotoxic or ophthalmic manifestations may be lacking, but in the absence of both one could not make a diagnosis of Graves' disease.

I have been particularly interested of late in those patients who complain of their eyes first. Often they go first to the ophthalmologist and sometimes he fails to appreciate that they actually have Graves' disease, not a local ophthalmic disorder. Early this month, for example, I saw a doctor's wife who last May thought she had eye strain and consulted an ophthalmologist. He told her she had muscle trouble with poor convergence. His observation was correct, but

\* After this lecture had been delivered at Nashville, Dr. Barney Brooks told me that I had ignored the action of the lids. He believed that one cannot have lid retraction without some degree of protrusion, that the inevitable effect of a widened aperture is some forward movement of the globe due to diminution in the restraining action of the lids. Soon afterward, Dr. David G. Cogan, at the Howe Laboratory of Ophthalmic Research, was good enough to make some observations on normal people (including myself). By means of ophthalmometric measurements he found that when the lids are held open wide with a speculum there is no protrusion beyond the limit of accuracy of the instrument, which is 2 mm. Some of the subjects, however, during voluntary retraction showed protrusion of 3 mm. Cogan concluded that it does not appear from this small series that the lids exert an appreciable effect in retaining the normal eyeball in the orbit. The active contraction of the levator, however, may force the globe out slightly through pressure on orbital contents. In view of these findings I cannot believe that widening of the aperture is an important factor in the causation of the proptosis of Graves' disease.



apparently he failed to recognize that this could be Moebius' sign in Graves' disease. Soon after she developed goiter and thyrotoxic symptoms. Most of the patients who go first to the ophthalmologist or eye clinic come to fall within our special ophthalmopathic group, but not all.

As Graves' disease progresses the ophthalmic picture may develop in various ways. In addition to lid retraction, proptosis may appear and increase in intensity. It may be asymmetric, as in the case of lid retraction, but it is rarely completely unilateral. I have often seen patients who at first glance appeared to have but one eye sticking out. However, on careful examination, some abnormality, perhaps only lid retraction or a lid lag, could be detected on the other side. If it can be demonstrated that eye signs are bilateral, even though asymmetric, it serves to rule out such lesions as postorbital tumor or arteriovenous aneurysm of the cavernous sinus, which would give a purely one-sided

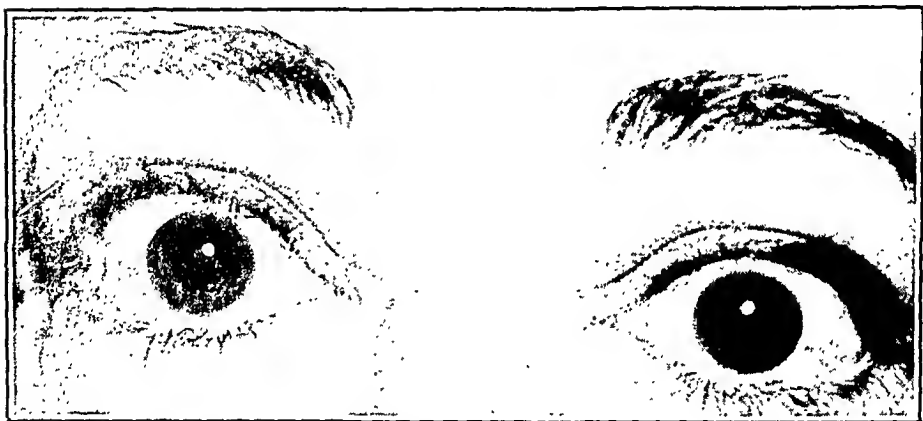


FIG. 4.—Graves' disease with development of progressive ophthalmopathy after thyroidectomy in a woman of 42 years. BMR -34 when picture taken. Lid retraction and proptosis very moderate, but edema and conjunctival injection marked. Chemosis present. Marked irritative symptoms, tearing, burning and so forth. (U. 252602.)

proptosis. Of other conditions than Graves' disease which can cause bilateral exophthalmos, we have encountered only skull deformities with shallow orbits, such as oxycephaly, Hand-Schüller-Christian's disease, rarely myasthenia gravis and possibly certain hypertension. In brief, the finding of bilateral eye signs of the Graves' variety is overwhelming evidence in favor of that diagnosis.

If the ophthalmic component of Graves' disease is of systemic origin, why, it may be asked, is it often so markedly asymmetric? To that I can give no adequate reply beyond saying that the evidence in favor of systemic origin is very convincing. As the eye lesion develops, asymmetry may either diminish or increase. I have seen cases in which first one eye was the more prominent, then later the other caught up with and even passed it.

In evaluating the ophthalmic status in any patients with Graves' disease, the congestive and dropsical phenomena are very important.

Compare, for example, the case of a patient, who had classic Graves' disease years ago and has permanent residual exophthalmos, with that of one who has just started on the course of what we call the special ophthalmopathic type. In the former, the pure proptosis may be very marked, but there will be little, if any, lid retraction, and no edema of lids or conjunctivæ, or congestion, and in all probability no ocular symptoms. The eye condition here is quite stationary and inactive. It is, in fact, an end-result. In the latter case, the picture is quite different. The eyes have a sore and swollen look. The actual proptosis may be less than in the former, but the lids are edematous, the conjunctivæ are injected and chemotic, and all these tend to make his general appearance much more impressive, or even alarming, than in the case first described. The patient with the latter picture will have plenty of ophthalmic symptoms: tears, smarting, photophobia and so forth. One patient said that his eyes felt like golf balls. In another

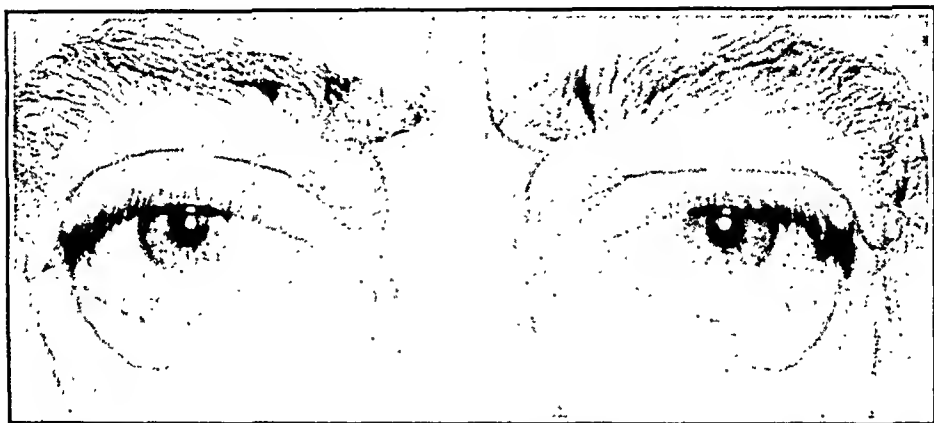


FIG. 5.—Graves' disease with development of a malignant type of ophthalmopathy 3 years after thyroidectomy for thyrotoxicosis, in a man of 54. BMR +5 off iodine—later to -15 on iodine. No lid retraction and therefore proptosis, which was of considerable degree, was obscured. Marked injection and chemosis of conjunctivæ. Later developed corneal ulcerations and required orbital decompression O.D. (U. 292087.)

patient the chief complaint may be the result of muscular weakness: diplopia or inability to fix near objects, difficulty in reading, and so forth. This type of ophthalmopathy represents an active process.

When Graves' disease is treated by partial thyroidectomy, the eyes often lose much of their staring appearance rather rapidly. The exophthalmos appears to subside. However, Soley<sup>63</sup> has shown that usually after operation actual measurement shows that, for a time, exophthalmos actually increases. The improved appearance is to be attributed to decline in lid retraction. In the classic type, however, ultimately the exophthalmos may decrease, although, as Galli-Mainini<sup>17</sup> has shown, it seldom completely disappears. Sometimes the eyes come to rest permanently in a markedly forward position, as in the case described earlier.

In those cases which fall in the special ophthalmopathic group, the eye condition becomes aggravated after operation and the progress of

the lesion may be such as to justify the appellation of malignant exophthalmos. Edema of the lids and chemosis of the conjunctiva become extreme. Ulceration of the cornea sets in, vision, and indeed the eyeball itself, is endangered. A truly terrible picture of bulging, inflamed eyes protruding through swollen lids is presented. The patient cannot close his lids, and this aggravates the inflammation through the irritating effect of drying of the cornea.

We have come to believe that when a hint that the ophthalmopathy may follow such a course is obtained before thyroidectomy is done, the most important indication for treatment is to refrain from removing the thyroid. It is significant, we believe, that in cases of malignant exophthalmos in which it finally becomes necessary surgically to decompress the orbit in order to save the eye, there has previously been a thyroidectomy in a very high percentage of cases. In other words, it is (with very few exceptions) only the post-thyroidectomy cases in

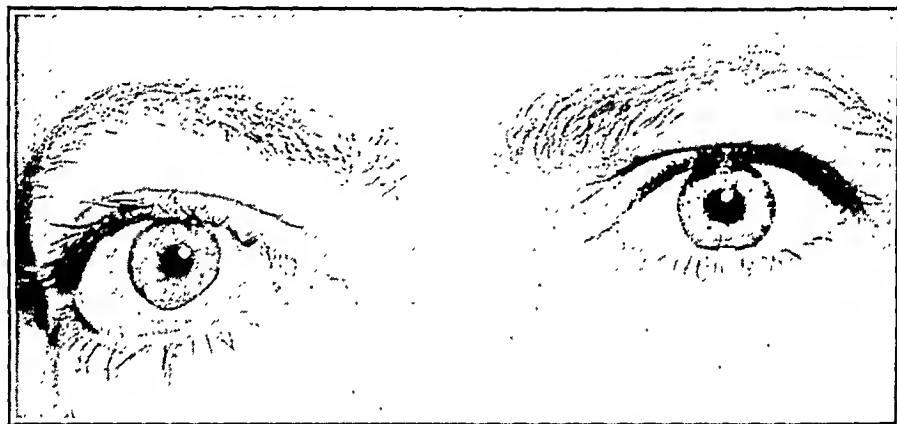


FIG. 6.—Asymmetric ophthalmopathy developing in a woman of 51 who had had thyroidectomy for Graves' disease 5 years earlier. She was also diabetic. At time picture was taken she was receiving iodine, thyroid and insulin. Her BMR was 0. No lid retraction, but moderate proptosis and extreme chemosis and conjunctival injection O.D. Her right orbit was treated by irradiation and she made a marked improvement. (U. 315242.)

which such serious eye conditions develop. Occasionally patients, who have had the disease in classic form and recovered with or without thyroidectomy, turn up some years later with a recrudescence which assumes the special ophthalmopathic type rather than the classic.

**Pathogenesis of the Ophthalmic Component.** Let us now consider the pathogenesis of the ophthalmic component, and its relation to that of the disease *in toto*. There are, of course, two main approaches: the study of the naturally occurring disease in man and the endeavor to reproduce it experimentally in other species. Also the problem can be divided into that of the local intra-orbital mechanism and that of what lies behind it.

On the former question, observations on humans are probably more valuable than experiments on animals. The difficulty with animals is that the mechanism of exophthalmos may be quite different from what

it is in humans. For example, as long ago as 1858, Müller<sup>45</sup> distinguished two groups of smooth muscles in the eyes of mammals, periorbital and palpebral. In some species the periorbital is so well developed as to constitute a slinglike muscle capable of pushing the eyeball outward on sympathetic stimulation or on the exhibition of sympathomimetic drugs (Brunton<sup>9</sup>). This has been called Müller's muscle. It is rudimentary or absent in man and plays no rôle in human exophthalmos.

In 1904, MacCallum and Cornell<sup>36</sup> produced exophthalmos in dogs by obstructing the outflow of blood through the orbital veins. This was followed by the development of edema of the orbital tissues which further enhanced the exophthalmos. Again it is unlikely, as these authors admit, that such a mechanism plays a rôle in human Graves' disease.

A better clue to the situation in man is provided by the observations of the English ophthalmologist, Moore,<sup>44</sup> in 1920. In a case of malignant exophthalmos he incised the conjunctiva and removed as much orbital fat as possible to relieve tension. He remarked that the fat "seemed edematous, and in particular the inferior, internal, and external recti muscles were exposed, for a considerable distance, and these, instead of being thin, flat, ribbon-like muscles, such as one becomes familiar with in squint operations, were greatly swollen fusiform bellies apparently from oedematous infiltration, not quite so stout as the last joint of one's little finger." So far as I know, Moore's is the first study of the pathology of the living in this condition. He also obtained an autopsy in 1 case of exophthalmic goiter and concluded that, since the exophthalmos remained marked after death, it could be due neither to sympathetic irritation nor to venous engorgement, but rather to edema of orbital contents, and perhaps increase in orbital fat. Thomson,<sup>67</sup> in 1924, also upheld the edema theory, saying "exophthalmos in exophthalmic goitre is due to a localized edema in the posterior part of the orbit, which, for some unknown reason, if sufficiently prolonged, is followed by connective tissue proliferation and permanent thickening of the orbital tissues." We might suggest that the edema, like that in the legs of patients with long-standing cardiac failure, becomes brawny. The German, Unverricht,<sup>68</sup> in 1925, also supported this theory.

A really important contribution to the pathology of the condition is that of Dudgeon and Urquhart,<sup>11</sup> in 1926. These investigators examined extrinsic eye muscle, heart muscle and skeletal muscle in 9 post-mortems on exophthalmic goiter patients. Lymphorrhages, great infiltrations with lymphocytes and some plasma and endothelial cells, were found in the muscles of 8 of the 9 cases. They were most marked in the ocular muscles.

The first extensive observations of the pathology of the living in the exophthalmos of Graves' disease are those of Naffziger,<sup>46</sup> who in 1931 devised the operation of orbital decompression. On opening the orbit through the cranial cavity, Naffziger found the orbital contents bulged, due entirely, he thought, to a great increase in the size of the

extrinsic muscles (3 or 4 times their normal volume). Microscopically, the muscles showed edema, round cell infiltration often with a perivascular pattern, loss of architecture, increase in fibrous tissue with areas of hyalinization and fragmentation, and destruction. Friedenwald<sup>13</sup> confirmed Naffziger's findings in 1932.

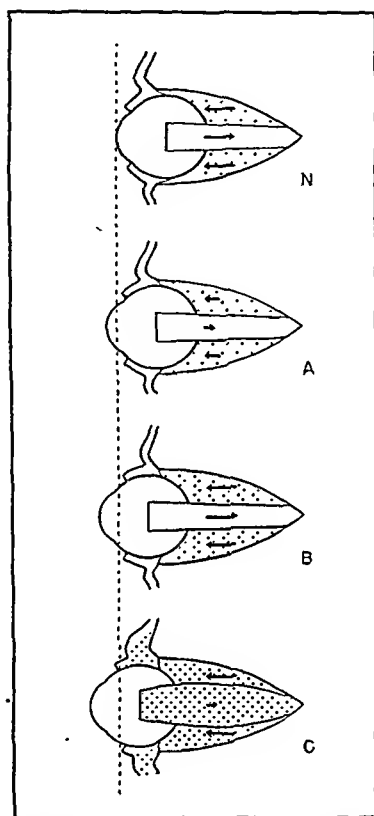


FIG. 7.—Galli-Mainini's schemata representing the mechanism which may be involved in exophthalmogenesis. "The lids, the eyeball, one muscle and the intra-orbital tissue are represented. The arrows indicate the direction of pressures and tensions. The dots represent the amount of fluid in the tissues. In Schema N is shown the situation in health. The pull of the muscle is of the same magnitude as the tissue pressure and balances it, so that the position of the globe is that found in normal persons, indicated in these schemata by the interrupted line. Schema A shows the first step in the development of exophthalmos. The muscle has weakened, exerts less tension, indicated by the shortened arrow. The tissue pressure therefore forces the eyeball forward. As this happens tissue pressure falls and fluid passes from the capillaries to tissue to occupy the space, until a new equilibrium is reached with the muscles stretched and the eyeball protruding—Schema B. In Schema C is shown the situation in progressive exophthalmos in which a weakened and degenerate muscle cannot withstand the tissue pressure and in its turn becomes edematous, as do the lids and surrounding tissues."

Thus it seems established that a part of the immediate cause of exophthalmos in Graves' disease is swelling of the orbital contents. In the malignant cases, swelling of the muscles may be the chief factor. In others, edema of the other orbital contents may play a rôle.

That the whole phenomenon of proptosis may not be due to increased push upon the globe, however, is suggested by Galli-Mainini.<sup>17</sup> He points out that there is also the factor of decreased pull. "The eye-

ball," he says, "fits the orbit somewhat as a cork does a bottle, and it is maintained in position by the action of two pressures working in opposite directions. One of these is the pressure exerted by the tension of muscles, which tends to pull the eyeball backward; the other is the tissue pressure of the orbital contents, which tends to push the eyeball forward. The two are in such balance in health as to maintain a constant volume of orbital content, and at the same time to secure normal movement of the globes and circulation of the orbit."

The cork and bottle simile is good, and one should perhaps further specify a bottle of champagne. Here we have a cork held in by wires against a high pressure within the bottle. Cut the wires and out comes the cork. Weaken the muscles and out comes the eyeball.

That the ocular muscles may be weakened in Graves' disease, from the kind of pathologic change found by Moore, Dudgeon and Urquhart, Naffziger and Friedenwald, seems a fair inference. Also there is abundant evidence that generalized weakness of striated muscle is characteristic of the malady.<sup>2,5,33,37</sup> That a tendency to water retention also exists in Graves' disease seems likely in view of the frequent occurrence of moderate edema without evidence of heart or renal failure. Why in a general condition should edema formation be chiefly manifested locally in the orbit? This may be because it is only in the orbit that we have the cork and bottle set-up. The combination of increased tissue pressure and decrease in the force opposing it (the pull of the extrinsic muscles) is only to be found there. Galli-Mainini visualizes the sequence of events as follows: A weakening of the muscles upsets the equilibrium between the muscle pull and tissue push of health. The globe moves forward. As this happens, tissue pressure falls and fluid passes from capillaries to tissue to occupy the space, until a new equilibrium is reached with the muscles stretched and the globe protruding. If this situation is stabilized we have the sort of permanent inactive residual exophthalmos which I have described. On the other hand, it may become progressive and as the muscles weaken they cannot withstand the tissue pressure and in turn become edematous, as do the lids and surrounding tissues. According to this concept, edema of the lids, and such phenomena as accompany it (chemosis and injection of the conjunctiva), would always denote an active or progressive process, and absence of them, as Haines<sup>20</sup> has pointed out, would indicate an inactive or receding condition.

On the purely experimental side we have a number of leads. Many investigators report the production of exophthalmos in animals with extracts of the anterior pituitary. The first of these, I believe, was Schockaert,<sup>60</sup> who produced thyroid hyperplasia and exophthalmos in young ducks, in 1931. I remember his telling us about it on a visit to our laboratory and causing much excitement thereby. A year later, Loeb and Friedman<sup>35</sup> obtained exophthalmos in guinea pigs by injecting an acid extract of anterior pituitary of cattle. As this exophthalmos receded if injections were discontinued and under narcosis or postmortem, it differed from the exophthalmos of Graves' disease in humans. Marine and Rosen<sup>41</sup> next showed that exophthalmos can be produced by TSH injection even more easily in thyroidec-

tomized guinea pigs than in intact ones. In 1934, Friedgood<sup>14</sup> observed that TSH-induced exophthalmos in guinea pigs became worse during a refractory period, and was especially well marked in animals in which a low basal metabolic rate developed. This finding fits very well with what we observe clinically.

Smelser,<sup>61</sup> in 1936, confirmed Marine and Rosen's finding that thyroidectomy enhances the exophthalmos induced in guinea pigs by TSH injections. He also showed that if one side be sympathectomized in advance the exophthalmos is less on that side. This might not hold in the human. Smelser,<sup>62</sup> furthermore, found that the orbital fat and connective tissue of his exophthalmic pigs was edematous and the muscles were increased in size. Paulson,<sup>48</sup> of the Mayo Clinic, in 1937, reported similar findings in the orbit and in the extra-ocular muscles, changes similar to that described by Naffziger in humans. Paulson found no changes in other skeletal muscles except the orbicularis oculi in which, in a few instances, degeneration of the fibers was noted. In 1939, Paulson<sup>49</sup> published further work showing the production of marked retroglobal edema in TSH-injected thyroidectomized guinea pigs, which he believed responsible for the ocular protrusion. He also, this time, obtained definite degeneration of skeletal and cardiac muscle. Iodine had no effect on the development of exophthalmos, although it did lessen the thyroid hyperplasia induced by TSH. He concluded that iodine acts on the thyroid gland and is not directly inhibitory or antagonistic to TSH. We entirely agree with this interpretation. In 1940, Aird<sup>1</sup> showed that after several months of injection of TSH the exophthalmos of guinea pigs persists despite cessation of injection and after narcosis or death, attributing this to the enlargement of the muscles. In discussion of Aird's paper, Marine pointed out that there were two types of exophthalmos: that without and that with organic change in the orbit. The second has not been produced in the rabbit, only in man and the guinea pig, and for the most part after thyroidectomy.

Marine<sup>40</sup> believes that in addition to the anterior pituitary, the gonad plays an important rôle in the production of exophthalmos. For example, castration of male rabbits caused an existing exophthalmos to regress. The exhibition of testosterone propionate to these same rabbits caused the exophthalmos to recur. Since these observations were made on rabbits, they may be quite inapplicable to man. However, it is of interest to note, in this connection, that those cases of Graves' disease which we have called the special ophthalmopathic type, are relatively more numerous in males than are those of the classic type. Marine also believes the adrenal cortex may be involved in the endocrine hookup of Graves' disease, though the connection seems obscure.

In the light of all these various studies on the relation of TSH to exophthalmos, it is interesting to recall that Cannon<sup>10</sup> and his co-workers, back in 1915, produced in 4 cats a syndrome very like human Graves' disease by joining the phrenic nerve with the cervical sympathetic. These cats developed exophthalmos and hyperthyroidism. They also had hyperplastic adrenals. The authors concluded that the thyroids of their animals had become overactive from direct ner-

vous stimulation, but the weight of modern evidence favors the interpretation that actually it was the anterior pituitary which was nervously stimulated and the thyroid, in turn, humorally by the pituitary. Such a theory accounts for the adrenal hyperplasia also.

Of late there has been much interest in the effects of cyanates upon the thyroid in man. It has been found by several observers,<sup>6,32,55</sup> including my colleagues and me, that patients, treated for a long time with potassium sulfocyanate for hypertension, may develop a syndrome characterized by hyperplastic goiter with hypofunction. In 1 of our cases there was also slight, but definite, exophthalmos. Furthermore, thyroid hyperplasia has been produced in animals by other sulfur-containing substances such as thiourea, thiocarbamide and the sulfonamides.<sup>3,4,38,39</sup> Rawson has very recently been treating rats, in our laboratory, with sulfathiazole in order to produce hyperplastic goiter. He gained this objective satisfactorily, but also found that his rats developed very definite exophthalmos which persisted after death. It is not clear precisely where such agents impinge on the pituitary-thyroid axis, but the weight of evidence is that it is on the thyroid directly, imposing some obstruction to the completion of the elaboration of thyroid hormone. The organism thus becomes hypothyroid, which serves as a stimulus to the pituitary to increase its output of TSH, which, in turn, causes the thyroid to become hyperplastic and the eyes to become protruded. The hyperplasia of the thyroid, however, is ineffective because the obstruction to completion of thyroid hormone persists.

**Summary.** What can we conclude about the nature of Graves' disease from the various bits of evidence which I have cited? Not much, perhaps, but yet something.

Seemingly the malady is determined, to some degree, by inherited constitution, plus, very likely, some environmentally imposed stress which the given constitution cannot take without departing from the healthy state.

Hyperplasia of the thyroid seems to be a characteristic feature. Usually, but not always, there is production of hyperthyroidism.

The behavior of the thyroid is very likely secondary to humoral stimulation from the anterior pituitary. The overactivity of the pituitary may well be due to a nervous impact reaching it *via* the hypothalamus.

The response to whatever the stimulus be, which sets off the disease, may follow a different course in one individual from that in another.

The ophthalmic portion of the disease is both interesting and mysterious. It is of great importance from the point of view of therapeutics.

It is fairly evident that swelling of the extraglobular orbital contents, especially the extrinsic muscles, is a major immediate cause of exophthalmos. Weakness of the muscles, with decreased back pull, is probably another—indeed, the latter may be the first to become operative.

The cause of these local changes, and what determines their progression, or recession, is not so clear. Certainly they are not the result of thyrotoxicosis *per se*. The thyroid hormone is diuretic in action. It should decrease swelling, not cause it. Considerable evidence supports



the view that an excess of thyrotropic hormone has something to do with the ophthalmic pathogenesis, but the relationship is probably not a simple one.

Why some cases follow what I have defined as a classic course, others an ophthalmopathic one, may be related to differences in the response of the thyroid cells to the action of TSH. In the classic type, TSH stimulates the cells, and they produce an excess of their hormone. In this process, TSH is inactivated and, as Rawson has shown, is excreted in that form in the urine. In the ophthalmopathic type, TSH is not inactivated to the same degree, the production of thyroid hormone is not increased and TSH is excreted in its active, as well as inactive form in the urine. At the same time, excess of TSH aggravates the ophthalmic situation.

I have said but little of treatment, but in closing I urge you, in planning treatment in this disease, to keep the morbid mechanism, in all its known aspects, in mind. Particularly, realize that the best therapeutic program may be quite different in one case from what it is in another. While subtotal thyroidectomy may be the best treatment we have at present for many patients, in other cases it is not only not helpful, but very likely harmful. To accept routine subtotal thyroidectomy as good enough treatment and not seek for better, is not a truly scientific attitude. This is the thought I should like chiefly to leave with you.

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## TREATMENT OF ADDISON'S DISEASE WITH PELLETS OF DESOXYCORTICOSTERONE ACETATE\*

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DESOXYCORTICOSTERONE is the only adrenal cortical steroid which has been prepared synthetically, and hence is the only pure cortical principle available in a quantity sufficient for therapeutic use. Compared to the other adrenal steroids desoxycorticosterone possesses a high

\* The desoxycorticosterone acetate (cortate), both in the form of pellets and dissolved in oil, was generously provided by the Schering Corporation through the courtesy of Dr. W. H. Stoner.

potency in maintaining the life of adrenalectomized animals, and exceeds all others in its capacity to stimulate retention of sodium chloride.<sup>12</sup> It has the practical advantage of being less expensive than cortical extract. One of its disadvantages, however, is the danger of overdosage. Edema, hypertension, cardiac failure, and paralytic states have been observed when excessive quantities of the compound were administered.<sup>5,7,18,23,28</sup> Furthermore, desoxycorticosterone lacks any appreciable capacity to influence carbohydrate metabolism,<sup>16,17,27</sup> and it fails to restore certain physiologic defects which follow adrenalectomy in animals.<sup>9,24</sup>

Despite its shortcomings this crystalline compound offers the unique advantage that it may be compressed into pellets, which, when implanted subcutaneously, give up the hormone to the tissues slowly and evenly so that therapeutic effects may be sustained for many months. The convenience to the patient, the absence of fluctuations in hormone absorption associated with injections, and the avoidance of all the uncertainties of self-medication are obvious points in favor of a preparation administered in pellet form. Moreover, Thorn, Greif, Coutinho, and Eisenberg<sup>29</sup> have pointed out that desoxycorticosterone when given in this manner is more effectively utilized than when given by any other method. Recently Thorn, Dorrance, and Day<sup>30</sup> have reported a series of 148 patients in which the results of treatment with pellets were, in general, very favorable. Sixty-one of these patients were under the direct observation of the authors. A number of other favorable reports have also appeared,<sup>2,3,8,10,11,13,14,20,22,23,25</sup> but these publications, for the most part, present data on only 1 or 2 patients who were observed for a relatively short period of time.

Pellet preparations of desoxycorticosterone acetate are not as yet available for general clinical use. The implication, therefore, exists that evidence pertaining to the desirability of pellet therapy is incomplete. Objections to such therapy might include not only an uncertainty as to its efficacy but also a fear of the effects of overdosage which cannot be alleviated without surgical removal of the pellets. In addition to questions of efficacy and hazard, clarification is also needed in relation to problems bearing on the type of pellet to be used, the number of pellets which should be implanted, their rate of absorption, and their maximum effective life.

The present report includes observations made on 7 patients with Addison's disease who have received treatment with pellets of desoxycorticosterone acetate. The diagnosis was established in each case by the presence of a typical clinical picture of the disease along with chemical studies which usually included a chloride excretion test<sup>1</sup> or water test<sup>21</sup> (Table 1). In 3 patients who have died there was confirmation of the diagnosis at autopsy.

**Method of Insertion of Pellets and Number Implanted.** Two types of pellets have been used. The first was flat and disk-like, measured approximately 3 x 9 mm., weighed 135 to 150 mg., and had a surface area of approximately 200 sq.mm. The other type of pellet, which is the one being used exclusively at present, is in the shape of an elongated

cylinder, measures 3.2 x 8.2 mm. and weighs 75 mg. Its surface area is 100 sq.mm. and volume 65 c.mm.

TABLE 1.—DIAGNOSTIC DATA

	Age	Sex	Concentration of Cl chloride excretion test (mg. per 100 ec.)	Water test		17 keto-steroids mg. per day	Electro-cardiogram	
				Night volume (ec.)	Maximum morning volume (ec.)			
Case 1 (A. G.)	35	M	...	500	40	2	5	Normal
Case 2 (F. G.)	62	F	...	..	..	..	2	Normal
Case 3 (G. H.)	47	F	205	..	..	..	..	...
Case 4 (G. L.)	35	F	346	197	50	3	5	Prolonged Q-T interval
Case 5 (J. B.)	28	M	...	435	85	5	5	Prolonged Q-T interval
Case 6 (M. W.)	30	F	...	300	50	5	3	Prolonged Q-T interval
Case 7 (R. P.)	33	M	274	1050	375	6	9	Prolonged Q-T interval

The pellets were inserted in the infrascapular region through an incision about 1 cm. long. Pockets radiating in various directions were made with a hemostat beneath the skin in the subcutaneous tissue. The pellets were then gently placed in position with smooth pick-up forceps. As many as 6 pellets could easily be inserted through one incision. There were no infections and in no cases were pellets spontaneously extruded. When the old pellets had been in place long enough to have undergone considerable absorption, they were removed, weighed, and new pellets inserted. There was usually very little tissue reaction around the pellets at the time of removal. A thin fibrous capsule, approximately 0.2 mm. thick was present and there was a variable degree of cellular infiltration just outside of the capsule.

Thorn has recommended that before pellets are implanted for the first time and at successive reimplantations the requirement of the patient should be determined by a preliminary trial with daily injections of the compound in oil.<sup>30</sup> He has calculated that one 125 mg. pellet should be inserted for every 0.4 to 0.5 mg. of hormone required by daily injection. Inasmuch as the pellets used in the present series of cases were not identical with those employed by Thorn, this calculation would not be applicable. A precise estimate of the optimum dose of this compound for a given patient is difficult to determine because a definite clinical or chemical end-point of response is lacking. Very small doses of hormone may be adequate to maintain normal electrolyte and water metabolism and yet not impart a satisfactory feeling of well being or restore muscular strength and endurance for work. In some patients an increase in the dose will bring about a substantial increase in subjective improvement, but in others signs of overdosage intervene and this end is not attained. On the other hand, many patients are well controlled and do equally well on doses of hormone varying as much as two-fold in amount.

In view of the difficulty encountered in determining precise dosage and in order to eliminate a rather impractical period of prolonged preliminary observation the patients in this series were implanted with pellets immediately after the period of diagnostic study. If within a few weeks or months there was not a degree of improvement which

seemed satisfactory, additional pellets were inserted. The optimal dose was considered to be that amount of hormone which, without signs of overdosage, maintained normal levels of plasma chloride, blood urea nitrogen, hematocrit, and blood pressure, and which gave a maximum degree of subjective improvement with moderate gain in weight. The initial dose was one or two 150 mg. pellets or two to four of the 75 mg. size. The maximum number eventually implanted in any patient to date has been four of the type weighing 150 mg. and six of the kind weighing 75 mg.

After leaving the hospital the patients were allowed free choice of food. The amount of salt ingested was derived only from that which was intrinsic in the foodstuff plus that which they chose to add to suit their tastes. This total varied from 8 to 12 gm. of NaCl per day as estimated from urinary excretion of chloride while salt intake and output were presumed to be in balance. When the pellets were nearing the final stages of dissolution weakness and anorexia sometimes appeared. These symptoms were temporarily controlled with extra salt until the time when it was deemed advisable to insert new pellets. In several instances recently, new pellets were implanted at the end of 9 or 10 months without removal of the old ones. Signs of overdosage did not appear.

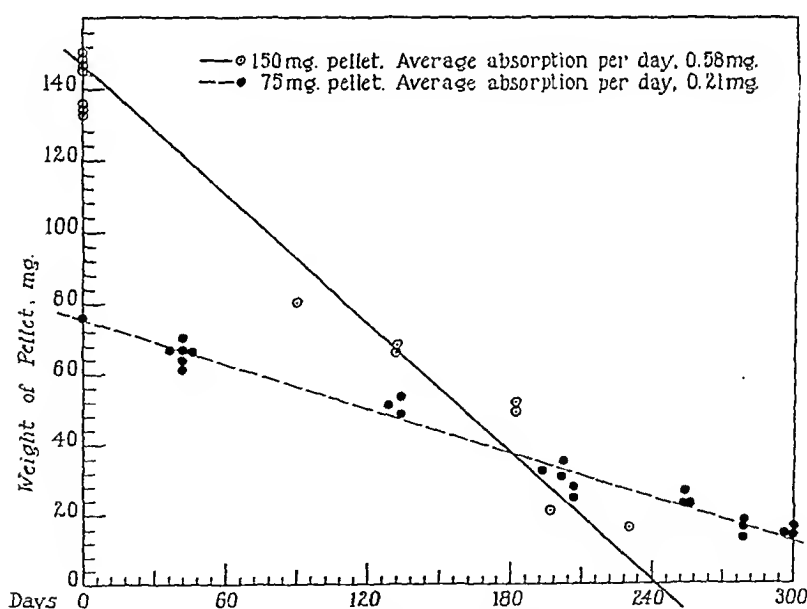


FIG. 1.—Rate of absorption of pellets.

**Rate of Absorption of Pellets.** If the weight of the pellets, after variable periods of absorption, is plotted against the time elapsing after implantation one obtains a distribution which tends to follow a straight line (Fig. 1). This indicates that the daily rate of absorption tends to be constant and that it does not decrease materially even after a pellet has become greatly reduced in size. This most fortunate

characteristic of pellet dissolution in both patients and animals has been observed previously not only with desoxycorticosterone but with other hormones as well,<sup>4,6,26</sup> and may probably be ascribed to a progressive roughening of the surface of the pellet along with an increase in permeability. In Figure 1 it may be seen that the daily rate of absorption of the large pellet averages 0.58 mg., and the maximum effective life is about 7 months. The daily absorption from the small pellet averages 0.21 mg. and the effective life may be estimated at 9 to 10 months. The rather small scatter of the individual points from the line representing the average rate of decline in pellet size indicates a reasonably constant rate of absorption from patient to patient and pellet to pellet. The effective life of a pellet is influenced by its weight, surface area, density and shape. By proper adjustments of these variables the maximum life of 10 months for a pellet might be materially lengthened. That pellet weight alone is not the sole factor is evidenced by the observation that the pellet weighing 150 mg. approaches complete absorption sooner than the one which weighs 75 mg.

TABLE 2.—SUMMARY OF THE EFFECTS OF PELLET THERAPY

	Case 1 (A. G.)	Case 2 (F. G.)	Case 3 (G. H.)	Case 4* (G. L.)	Case 5 (J. B.)	Case 6* (M. W.)	Case 7* (R. P.)
Duration of pellet treatment (mos.)	40	13	4	26	15	11	7
Clinical improvement	Excellent	Poor (death)	Poor (death)	Good	Excellent§	Good	Excellent
Overdosage symptoms	No	Yes	No	No	No	No	No
Hypoglycemia	Yes	Yes	Yes	No	Probably	No	No
Hematocrit:							
Before treatment	...	...	45	...	42	35	...
After implant	33	32	30	38	34	33	38
Blood urea nitrogen:							
Before treatment	36	17	47	10	20	12	...
After implant	10	10	15	9	6	15	12
Plasma chlorides (m.eq.):							
Before treatment	70	96	99	103	93	99	103
After implant	100	103	102	108	102	105	102
Blood pressure:							
Before treatment	85/60	100/60	78/50	110/70	75/50	90/60	105/70
After implant	105/70	110/60	98/60	115/70	90/50	105/65	115/75
Heart size:							
Before treatment	0.39	0.40	0.39	0.33	0.32	0.38	0.39
After implant	-13	-14	-12	-27	-15	-3	-1
After implant	0.42†	0.48	0.40	0.39	0.37	0.39	0.40
After implant	-2‡	-4	-8	-13	-8	+4	+1
Weight gain on treatment (lb.)	6	7	1	8	12	6	9
Number of 75 mg. pellets considered adequate for maintenance	4	...	...	6	6	4	4

\* On salt therapy when first seen.

† Cardiothoracic ratio.

‡ Heart diameter as % of normal (Ungerleider and Gubner<sup>21</sup>).

§ Died after an appendectomy.

**Clinical Results.** Four of the 7 patients are living and in all cases the symptoms of the disease are under reasonably good control. One patient is employed as an animal caretaker, 2 are doing factory work, and 1 assists with housework in her home. Three patients have died. Two of the fatal cases could not be controlled by pellets or by injections of desoxycorticosterone acetate in oil. The 3d had been well controlled by pellets but died recently after an appendectomy. Some of the specific therapeutic results which were observed in the various patients are listed in Table 2. The various values which were obtained after treatment are expressed as the averages of several determinations

taken while the patients were under the influence of a dose of hormone which seemed to be optimum. The hematocrit was consistently low in all patients after pellet therapy had been started. The values, in fact, were distinctly lower than normal. It is highly probable that this depression in cell volume was due to anemia rather than to excessive hemodilution. Blood urea nitrogen remained low in all cases except in Case 3, where it rose just before death. Plasma chlorides and blood pressure were easily maintained in all patients. The cardiothoracic ratio rose in all cases. Inasmuch as the cardiac diameter of the patients before treatment tended to be distinctly smaller than the normal average, this rise was not due to abnormal dilatation. In Case 2, however, during a period when signs of overdosage were present, cardiac enlargement was well enough marked to be considered abnormal (see case report below). McGavack has advocated that treatment be regulated by observing changes in cardiac size.<sup>19</sup> All patients slowly gained weight. In Case 3 this was minimal, however, and in Case 2 there was an eventual decline even in the presence of edema. Patient No. 1 has shown an appreciable diminution in the pigmentation of his skin and mucous membrane. The others have shown no change.

**Case Studies.** CASE 1 (A. G.). This patient was first admitted in 1938 because of an asthenia which was so profound that he appeared almost completely paralyzed. Buccal pigmentation and the usual symptoms of Addison's disease were noted. After an infusion of 0.9% sodium chloride there was dramatic improvement and subsequently he was maintained in fairly good condition with a total daily salt intake of 20 gm. On January 30, 1940, one 150 mg. pellet of desoxycorticosterone acetate was implanted in the left subscapular region. Urine analysis revealed that his voluntary salt intake with food was 10 gm. per day. Within 4 to 5 months the left breast became enlarged and tender and upon palpation a well defined circular mass of tissue measuring 3 to 4 cm. in diameter could be made out just underneath the region of the nipple. A new pellet of similar size was inserted in the right subscapular region on August 14, 1940. The breast enlargement gradually subsided and has never been noticed again in this patient or in any others in the series. On April 4, 1941, what was left of the old pellets was replaced with two 75 mg. pellets. Two months later the patient was admitted because of a sore throat and vomiting. For 24 hours before admission the food intake had been almost nil. There were symptoms of hypoglycemia and blood sugar was 51 mg. per 100 cc. The signs of hypoglycemia promptly disappeared after administration of glucose intravenously and within a few days when the infection had subsided he was again asymptomatic. In September, 1941, he obtained a job in the animal room of the hospital, which he has held until the present time.

In June, 1942, after a Saturday morning's work he went out for a few "beers," ate no lunch, and after arriving home in the early evening, went to bed without eating. The next morning he was found unconscious on the floor of his bedroom. On admission to the hospital he was comatose but restless. The skin was cool and moist, blood pressure was 110/90, temperature 34.6° C., pulse 60. Unfortunately, no blood was taken for analysis, but his recovery of consciousness a few minutes after administration of glucose intravenously is highly suggestive of another episode of hypoglycemia. There was a sharp rise of temperature to 40° C. and patchy consolidation was discovered in one lung. He received 100 cc. of cortical extract and 20 mg. of desoxycorticosterone acetate in oil during the next 24 hours and after continuous vigorous treatment he completely recovered. The pneumonia was considered to be of

the "virus" type. With four 75 mg. pellets implanted he is now back at work with no symptoms whatever.

CASE 2 (F. G.). A 62-year old woman showing typical signs of Addison's disease was admitted in April, 1940. A chloride excretion test could not be completed because of extreme weakness, nausea, and a fall in plasma chlorides to 85 m.eq. Two pellets weighing 135 mg. each were implanted and she was discharged on a total dietary salt intake of approximately 10 gm. per day. Her weight on discharge was 110 pounds. After a month at home she reported that her strength and appetite were still poor although she had gained 3 pounds in weight. Blood pressure was 105/60, plasma chlorides 103, and blood urea nitrogen 9.8. Salt intake was increased to a total of 15 gm. per day. Two weeks later she awoke in the morning with edema of the hands, face and feet, orthopnea, dyspnea, cough and dilated neck veins. Her blood pressure was 135/70. All symptoms of overdosage disappeared within 24 hours after she was deprived of salt. With a total of 10 gm. of salt in the diet she remained weak and her appetite was poor. Her weight rose to 117 pounds and there was continual slight pitting edema of the ankles. She now complained of pain in various muscles and joints. Reduction of daily salt intake to a total of 5 gm. was followed by disappearance of the edema and fall of blood pressure to 115/60, but there was no change whatever in her subjective symptoms. In September, 1940, a generalized vesicular eruption accompanied by fever appeared and persisted for 1 week. Anorexia was now accompanied by spells of nausea and vomiting. In October, 1940, it was decided to increase her hormone dosage and markedly restrict her salt intake in the hope that physiologic effects of the hormone other than those controlling electrolytes might be brought into prominence. Four 150 mg. pellets were therefore implanted and the only source of salt allowed was that in her unsalted food—probably 3 gm. a day of NaCl. Slight edema of the face and ankles appeared but her weight fell to 110 pounds. Blood pressure gradually rose to 135/75 and in January, 1941, she began to experience substernal distress on exertion. The cardiac silhouette had increased to an abnormal size so that the cardio-thoracic ratio now measured 0.53. The electrocardiogram, which had previously been normal, now showed a left axis deviation and prolonged Q-T interval. There was no deviation of the S-T segment during exercise. After removal of one pellet the anginoid symptoms disappeared, but slight edema persisted. Potassium citrate, 3 gm. a day, caused no improvement or loss of edema but simply aggravated her feeling of weakness. In March, 1941, there was an episode of pleural effusion, pleural pain, and fever of 39.5° C. All dependent edema disappeared. Blood pressure was 145/80. The cardiac silhouette was extremely large but it was not certain whether this was due to pericardial effusion or cardiac dilatation. After 10 days the pleural process cleared and the heart shadow became normal. The pleural fluid produced no lesions when injected into guinea pigs. Two more pellets were removed and she was sent home with no therapy other than 15 gm. total salt per day. Her appetite grew worse, she became even weaker, there were severe muscle and joint pains and diarrhea. In May, 1941, desoxycorticosterone, 5 mg. a day, intramuscularly, was started. Her appetite failed entirely so that only a glass of milk was taken daily. One week after the start of injections she was seen at home in a semicomatose condition. The skin was cold and sweaty. Blood pressure was 110/60. There was no edema. A blood sample showed a urea nitrogen of 5.7 mg. per 100 cc., plasma chlorides 111 m.eq., hematocrit 26%, and blood sugar "too low to read." Death occurred several hours later in a convulsion. Autopsy showed severe bilateral atrophy of the adrenal cortex.

This patient did poorly on a wide range of dosage of desoxycorticosterone. Strength, appetite, and body weight were not maintained. Overdosage effects were produced at higher dose levels. Although plasma chlorides, blood urea nitrogen, and blood pressure were normal shortly before death, the hormone failed to maintain the blood sugar



level when food intake had declined, and death ensued from hypoglycemia.

CASE 3 (G. H.). A 47-year old colored woman was admitted in February, 1941, with the usual signs and symptoms of the disease. In March, 1941, two 75 mg. pellets were implanted. It was determined that the diet which she consumed contained a total of 5 to 8 gm. of salt per day. She became somewhat stronger, her appetite improved, but her weight remained unchanged. In July, 1941, she gradually lost 8 pounds of weight, grew weak, and lost her appetite. At this time she was running a fever of  $38^{\circ}\text{C.}$ , blood pressure was 108/60, hematocrit 28%, plasma chlorides 98 m.eq., and blood urea nitrogen 15. Her salt intake was increased to a total of 15 to 20 gm. a day. There was no improvement and she stopped eating entirely. Blood sugar was 44 mg. on admission to the hospital at this time, chlorides 95 m.eq., blood urea nitrogen 23 mg. per 100 cc., hematocrit 26%, blood pressure 105/70. She was treated with parenteral glucose, saline and 10 mg. desoxycorticosterone daily. Nausea and vomiting grew more severe, the neck became stiff, and a Babinski sign was demonstrable on the left. Her temperature rose to  $41.2^{\circ}\text{C.}$  and coma ensued. Shortly before death her blood pressure was 120/70, plasma chlorides 110 m.eq., blood sugar 133 mg. per 100 cc., and blood urea nitrogen 32.3. Autopsy revealed bilateral tuberculosis of the adrenal cortex and a few microscopic tubercles of the periaortic lymph nodes, liver, and lungs. The brain and meninges were normal.

Although microscopic lesions were demonstrable in several organs of this patient in addition to the involvement of the adrenal cortex, it is unlikely that tuberculosis was the cause of the high fever and the fatal outcome. Hyperpyrexia has been observed in cases of uncomplicated Addison's disease by Loeb.<sup>15</sup>

CASE 4 (G. L.). This 35 year old woman was admitted in April, 1941, with a 2 year history of the disease. During the preceding year she had received extra salt and some injections of desoxycorticosterone acetate in oil. Four 75 mg. pellets were implanted and the total salt ingested with the diet was 5 to 7 gm. per day. The number of pellets was later increased to 6. She has had no nausea and her strength is sufficient to do a full day of housework. Her physical endurance, however, is not quite equal to that which she enjoyed before the onset of her disease. Although she has gained in weight there has been no edema.

CASE 5 (J. B.). A 28 year old man admitted in January, 1942, complained of symptoms of the disease for at least 2 years. A chloride excretion test could not be completed because of nausea, vomiting, and a generally poor clinical condition which was precipitated by the test. With four, and later six 75 mg. pellets in place and a total daily dietary NaCl intake of 10 to 12 gm. he gained weight and was able to do hard physical work. There was an episode associated with a respiratory infection when he failed to eat for 24 hours and was found unconscious in bed in the morning. It may be presumed that the coma was due to hypoglycemia, inasmuch as recovery was complete within a few hours after a generous intake of fruit juices. Recently he entered the hospital with typical signs of appendicitis. Symptoms had been present for 48 hours. During the next 8 hours he was prepared with glucose, saline, 5 mg. of desoxycorticosterone, and 30 cc. of cortical extract, after which a gangrenous appendix was removed. During the first postoperative day he received 94 cc. of cortical extract along with glucose and saline. His temperature reached  $40.7^{\circ}\text{C.}$  but blood pressure never fell below 110/70. By the third postoperative day his condition seemed excellent. He was taking food by mouth and no parenteral fluids were administered. On this day 28 cc. of extract was given. He had received no desoxycorticosterone since before the operation. When seen at 5:15 A.M. by the nurse he seemed to be in excellent condition except for slight

nausea. At 6 A.M. he was found dead. It was learned that he had refused his supper and that his last food had consisted of a glass of milk at 3 P.M. Autopsy revealed adrenal cortical atrophy so severe that nothing unquestionably identifiable as cortical tissue could be demonstrated. The heart was somewhat dilated.

This case is an example of the relative ease with which certain patients may be maintained with pellets during the absence of stress but who present a difficult problem in the presence of intercurrent illness. The cause of death may have been hypoglycemia, but this is conjectural.

CASE 6 (M. W.). A 30 year old woman who had suffered from the disease for 9 months was admitted in April, 1942. After the insertion of four 75 mg. pellets she was sent home on a diet containing 6 to 8 gm. of NaCl per day. She is able to do factory work at present and considers her strength to be adequate although not quite up to her previous normal.

CASE 7 (R. P.). A 33 year old man, admitted in September, 1942, gave a 9 months history of Addison's disease. With four 75 mg. pellets in place he is now back at work as foreman in a steel mill and feels in every way as well as he had before the onset of his disease. His daily dietary salt intake totals approximately 10 gm.

**Comments.** It is important to note that it was evident very early in the treatment of the 2 patients who were poorly maintained by pellets that they were showing a poor response. Those patients whose initial response was good have continued to maintain a favorable course, and, except for complications due to intercurrent illness, they have not shown any important fluctuations in status.

The results of treatment in Case 2 may be considered totally unsatisfactory. Desoxycorticosterone therapy failed to maintain the appetite and body weight, and was not followed by a subjective feeling of improvement. It also failed to prevent a terminal hypoglycemia induced by fasting. In Case 3 the maintenance dose of hormone was probably too low, but more intensive treatment with desoxycorticosterone by injection failed to alleviate the severe gastric symptoms, the hyperpyrexia, and the coma which preceded death. It is noteworthy, however, that both of these patients died without going into the classical "crisis" of Addison's disease. Blood pressure did not fall and there was no evidence of depletion of electrolytes and fluid.

In the 5 remaining patients the pellet therapy may be considered to have produced very good results. All were able to work. One felt as well and as strong as he did before the onset of the disease, and the others not only felt reasonably well but were not seriously restricted in their activities. Patients Nos. 1 and 4, who had been maintained with salt therapy alone prior to treatment with pellets, felt stronger and in better general health while under the latter form of treatment.

The frequency with which hypoglycemia was encountered is notable. The present results add confirmation to previous reports that desoxycorticosterone does not repair the defect in carbohydrate metabolism which is associated with adrenal insufficiency. When desoxycorticosterone acetate is employed for maintenance of patients, one must, therefore, point out to the patient the danger of going without food.

It should be insisted that an adequate food intake be maintained even though the appetite fails. Hypoglycemic reactions are particularly apt to occur during infections and are probably attributable not only to an impaired appetite but also to the stress of the infection itself.

In the presence of a severe infection or any serious complicating illness the requirement for hormone is materially increased. The amount of hormone derived from the pellets becomes inadequate and intensive therapy with additional hormone is imperative. In such emergencies cortical extract in large doses should be given inasmuch as this preparation should theoretically provide cortical hormones with important physiologic properties not possessed by desoxycorticosterone. Additional desoxycorticosterone acetate in oil may also be given if necessary to assist in the maintenance of blood pressure, and electrolyte and water balance.

**Summary and Conclusions.** Five out of a series of 7 patients with Addison's disease were well maintained for 7 to 40 months by pellets of desoxycorticosterone acetate and were able to carry on work involving moderate physical activity. The other 2 patients were not satisfactorily controlled either by pellets or by the compound administered by injection. Both of these patients died from the disease. One patient died suddenly after an attack of appendicitis at a time when he seemed to be convalescing satisfactorily.

Patients under therapy with pellets of desoxycorticosterone acetate may easily be thrown into hypoglycemia by fasting. They are particularly vulnerable in the presence of an infection. Aside from its failure to restore normal carbohydrate metabolism, desoxycorticosterone is deficient in other respects. The symptoms of the disease cannot be controlled in all cases, and death cannot invariably be prevented no matter what dose is employed. It should be pointed out, however, that some cases of Addison's disease also do not respond to cortical extract.

On the basis of the present observations it is concluded that pellet therapy with desoxycorticosterone acetate is highly useful for the maintenance of most patients with Addison's disease. During infections or other conditions imposing stress the requirement for hormone is increased, and under these circumstances additional therapy, chiefly in the form of cortical extract, should be given.

The effective life of the 75 mg. pellet which is being used at present is approximately 9 to 10 months, the average daily absorption is 0.21 mg. per pellet, and the average number of pellets required is four to six. The rate of absorption is reasonably constant from patient to patient and pellet to pellet.

It has been possible to implant pellets without subjecting patients to a lengthy preliminary period of therapy with injections. In the one case in which removal of pellets was necessary because of overdosage the number of pellets had been deliberately increased for special reasons. Insertion of new pellets without removal of the old ones after the latter have been almost completely absorbed has not produced signs of overdosage.

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## THE USE OF FIBRINOGEN IN A RAPID METHOD OF DETERMINING CELL VOLUME

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THE determination of the volume of packed red cells has assumed an increasingly important rôle not only in the differential diagnosis of the anemias but in the evaluation of blood volume, shock, hemorrhage and dehydration. The volume of packed red blood cells "is an accurate index of the degree of anemia and is more easily and more accurately measured than even the red cell count. The cell volume constitutes the most useful single criterion of the degree of anemia available. It also serves to correct the sedimentation rate of blood for the influence of anemia or polycythemia."<sup>10</sup> The determination of the mean corpuscular volume, saturation index and volume index depends

in part upon the cell volume. The value of these studies in facilitating the diagnosis of the anemias has been established by Haden,<sup>6</sup> Osgood,<sup>7</sup> Wintrobe<sup>10</sup> and others. Finally, the cell volume determination has become an increasingly useful adjunct to the surgeon in recent years in measuring hydration and in the study of blood volume, hemorrhage and shock.

The volume of packed red cells may be determined by centrifuging a quantity of blood to which a suitable anticoagulant has been added. Centrifugation is carried out until the cell volume remains constant and no further packing occurs. The time of centrifugation has varied widely with different workers. It depends upon the type of hemato-crit, the speed of centrifugation and the length of the arm of the centrifuge. The recommended period of centrifugation varies from 30 minutes at 3000 r.p.m.<sup>10</sup> to 90 minutes at the same speed.<sup>4</sup> Haden<sup>6</sup> suggests 60 minutes at 2500 r.p.m. while other workers centrifuge the blood for 90 minutes at 2400 r.p.m.<sup>9</sup> The volume of packed red cells determined by centrifugation for 30 to 90 minutes is accurate to within 0.5%. The object of this report is to describe a method of determining cell volume in 5 minutes with an accuracy equal to the 30-to-90 minute method.

Fahraeus<sup>3</sup> in 1921 established the foundation for our present knowledge of the suspension stability of blood. He noted that fibrinogen decreased the negative charge on the red blood cells thus causing increased rouleaux formation and found that the increased sinking velocity of the red blood cells depended upon this increased agglutination or aggregation of the red blood cells. Maximum aggregation and the most rapid sedimentation of the red blood cells occurred in solutions of fibrinogen.

{The rapidity of sedimentation depends upon the size of the cell aggregates which in turn depends upon the fibrinogen content of the solution in which the red blood cells are suspended. The physico-chemical properties of fibrinogen affect the red blood cells in such a manner that the greater the plasma fibrinogen content the greater is the size of the settling red cell aggregates. The rate of settling of the red blood cells is directly proportional to the square of the radius of the suspended particles and follows Stokes' law of hydrodynamics. The size of the cell aggregates is thus the critical factor in the rate of settling of the red blood cells. }

We have observed in a previous communication<sup>5</sup> that the addition of electrophoretically pure fibrinogen to heparinized blood increased the sedimentation rate 300 to 800%, the increase being proportional to the amount of fibrinogen added. When 0.4 gm. per 100 cc. fibrinogen was added to whole blood and the sedimentation rate was determined by Wintrobe's method,<sup>10</sup> the cells settled so rapidly that they approached cell volume at the end of 1 hour. Since the rate of settling is proportional to the size of the cell aggregates and since the size of the cell aggregates depends upon the amount of fibrinogen added to the plasma, we investigated the use of fibrinogen in developing a rapid method for determining cell volume.

**Method.** Electrophoretically pure bovine fibrinogen, in the salt form prepared at the Armour Laboratories by the methods developed at the Department of Physical Chemistry, Harvard Medical School and supplied at the suggestion of Dr. Edwin J. Cohn, was used in all experiments. A neutral isotonic solution of fibrinogen was prepared by dissolving 0.31 gm. of the salt in 10 cc. of distilled water. Since the fibrinogen content is approximately 52% and the salt content 48% the final protein concentration of the solution was 1.6 gm. per 100 cc.

Heparin was the anticoagulant employed in all studies; 0.25 cc. of the fibrinogen solution (equivalent to approximately 4 mg. of pure fibrinogen) was added to 1 cc. of heparinized blood. The blood now containing an additional fibrinogen content of 0.4 gm. per 100 cc. was shaken gently to insure uniform mixing and was placed in a Wintrobe hematocrit tube and centrifuged for exactly 5 minutes. An interval timer was used to control accurately this period of centrifugation. Cell volumes were determined by reading the level of the cells and the upper level of the clear plasma in the hematocrit at the end of exactly 5 minutes of centrifugation. Determinations were made at 1500 r.p.m., 1800 r.p.m. and 2100 r.p.m. with an International Centrifuge Size 1, Type C, radius 17 cm.

Control studies were made simultaneously with heparinized blood containing no added fibrinogen and with blood containing an additional 0.25 cc. of homologous plasma or saline.

The cell volumes determined at the end of 5 minutes of centrifugation were compared with the cell volumes of the unaltered heparinized blood centrifuged for 60 minutes.

Sedimentation rates of the control blood and the blood with the added fibrinogen, plasma or saline were determined in each instance by Wintrobe's method.<sup>10</sup>

**Results.** The 5-minute method of determining cell volume was applied to 50 patients whose cell volumes varied between 17 and 68%. The cell volume determinations were between 20 to 30% in 6 of 50 patients, 30 to 40% in 10, 40 to 50% in 23, 50 to 60% in 7 and 60 to 70% in 4. The uncorrected sedimentation rates of the unaltered blood specimens varied between 0.5 and 64 mm. at the end of 1 hour (Table 1).

TABLE 1.—CELL VOLUMES OF BLOOD CENTRIFUGED FOR 60 MINUTES COMPARED WITH BLOOD CONTAINING ADDED FIBRINOGEN CENTRIFUGED FOR 5 MINUTES AT 1800 R.P.M.

Pt.	Cell volume		Sedimentation rate mm. in 1 hr.
	Control blood centrifuged 60 min.	Control blood + 0.4 gm. per 100 cc. fibrinogen centrifuged 5 min.	
K . . . . .	17.0	16.7	
B . . . . .	26.0	26.2	18.0
D . . . . .	29.0	29.0	34.0
H . . . . .	29.5	29.8	18.0
C . . . . .	33.2	32.8	33.0
A . . . . .	37.5	37.1	41.0
X . . . . .	43.2	43.2	9.0
I . . . . .	43.3	42.9	5.0
Z . . . . .	44.8	45.0	17.0
J . . . . .	46.5	46.5	42.0
Y . . . . .	48.0	48.0	5.0
F . . . . .	49.5	50.0	11.0
R . . . . .	58.0	57.5	.5
E . . . . .	58.1	58.0	2.0
G . . . . .	67.0	67.0	.5
P . . . . .	68.0	67.5	.5

Centrifugation for 5 minutes of blood containing the added solution of fibrinogen yielded cell volume determinations which were accurate to within 0.5% of the cell volumes of the unaltered blood centrifuged for 60 minutes at 1800 r.p.m. (Table 1). The mean deviation of the cell volumes obtained in 5 minutes was 0.24% in the 50 cases studied.

The accuracy of the 5-minute method is thus within the range of accuracy of the present methods requiring 30 to 90 minutes of centrifugation.

Table 1 demonstrates the accuracy of the method within a wide range of cell volumes (17 to 68%) and sedimentation rates (0.5 to 42 mm.). That this accuracy is independent of cell volume range or sedimentation rate is also illustrated in Figure 1 which compares the cell volumes of blood centrifuged for 60 minutes with blood containing added 0.4 gm. per 100 cc. fibrinogen centrifuged for 5 minutes at widely divergent cell volumes and sedimentation rates.

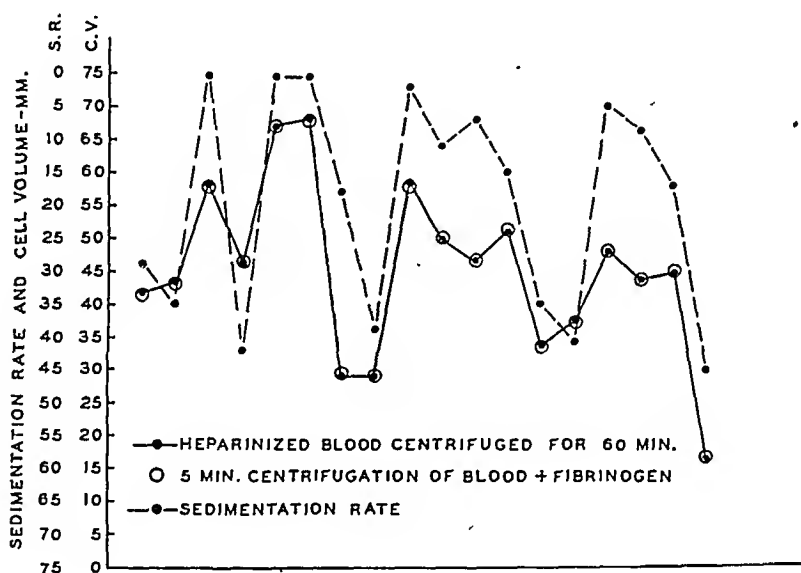


FIG. 1.—Comparative cell volume of blood centrifuged 60 minutes and blood with added fibrinogen centrifuged for 5 minutes.

The effect of the speed of centrifugation upon the 5-minute cell volume determinations is noted in Table 2. The most accurate results were obtained by centrifuging the blood at 1500 to 1800 r.p.m., the mean deviation from the 60-minute control being 0.26% and 0.17% respectively.

The 5-minute cell volume was identical with the 60-minute control in 4 of the 12 studies at both 1500 and 1800 r.p.m. The greatest single deviation was 0.5%. At 2100 r.p.m. the dilution effect became apparent and the 5-minute cell volumes were 1 to 2% lower than the 60-minute determinations. The mean deviation at 2100 r.p.m. was 1.25%. It may be concluded therefore that centrifugation should be carried out at 1500 to 1800 r.p.m. and that an error is introduced at higher speeds of centrifugation. Table 2 also demonstrates that the

accuracy of the 5-minute method is not affected by the range of cell volume or sedimentation rate.

TABLE 2.—EFFECT OF SPEED OF CENTRIFUGATION ON CELL VOLUMES DETERMINED IN 5 MINUTES

Control blood centrifuged 60 min. at 1800 r.p.m.	Control blood with added fibrinogen centrifuged for 5 min.			Sedimentation rate of blood mm. in 1 hr.	Sedimentation rate of control blood with added fibrinogen mm. in 1 hr.
	1500 r.p.m.	1800 r.p.m.	2100 r.p.m.		
46.9 . . . . .	47.2	46.8	45.2	25.0	48
33.2 . . . . .	33.2	33.0	32.5	31.0	61
32.2 . . . . .	32.3	31.9	31.5	60.0	67
22.8 . . . . .	22.8	22.6	22.2	46.0	73
52.0 . . . . .	52.0	51.7	50.5	18.0	53
36.5 . . . . .	36.2	36.0	35.5	12.0	55
44.0 . . . . .	44.3	43.8	42.7	10.0	47
22.8 . . . . .	22.8	22.6	21.8	64.0	76
63.0 . . . . .	63.2	63.0	61.5	1.0	33
67.5 . . . . .	68.0	67.5	66.0	0.5	22
69.0 . . . . .	69.5	69.0	67.5	0.5	14
44.0 . . . . .	44.5	44.0	42.8	19.0	47

Comparative 5-minute cell volume determinations of blood containing added fibrinogen, homologous plasma and saline indicate that accurate and consistent results are obtained with fibrinogen only (Table 3). This was demonstrated in the following manner: 0.25 cc. of the fibrinogen solution, homologous plasma and physiologic saline was added respectively to each of 3 tubes containing 1 cc. of blood, and the cell volume was determined by centrifugation for 5 minutes in the usual manner. Consistent and accurate cell volume determinations were obtained only in the blood specimens containing the added fibrinogen solution. The mean deviation from the 60-minute control was 0.2%, and the maximum deviation for this group was 0.5% (Table 3).

TABLE 3.—COMPARATIVE 5-MINUTE CELL VOLUMES OF BLOOD CONTAINING ADDED FIBRINOGEN, HOMOLOGOUS PLASMA, AND SALINE

Control blood centrifuged 60 min. at 1800 r.p.m.	1 cc. blood + 0.25 cc. fibrinogen solution centrifuged for 5 min.		1 cc. blood + 0.25 cc. homologous plasma centrifuged for 5 min.		1 cc. blood + 0.25 cc. physiologic saline centrifuged for 5 min.
	1500 r.p.m.	1800 r.p.m.	1500 r.p.m.	1800 r.p.m.	1800 r.p.m.
Cell volume					
22.8 . . . . .	22.8	22.6	21.8	20.4	25
32.2 . . . . .	32.3	31.9	30.5	28.5	35
33.2 . . . . .	33.2	33.0	35.0	34.2	36
36.5 . . . . .	36.2	36.0	36.7	36.0	40
44.0 . . . . .	44.5	44.0	48.0	42.2	49
46.9 . . . . .	47.2	46.8	48.5	46.7	51
47.0 . . . . .	..	47.2	..	51.5	53
52.0 . . . . .	52.0	51.5	55.5	50.0	57
63.0 . . . . .	63.2	63.0	67.5	65.0	68

Dilution of the blood with homologous plasma gave widely variable results. Centrifugation at 1500 r.p.m. for 5 minutes yielded cell volume determinations 0.2 to 4.5% higher than the 60-minute cell volumes in some cases and 1 to 1.7% lower in others. The mean deviation from the control was 2.3% with a maximum deviation of 4.5%. Centrifugation at 1800 r.p.m. yielded cell volume determinations 0.2



to 2.4% lower than the 60-minute control in some instances and 1 to 4.5% higher in others. The mean deviation was 2.1% and the maximum deviation 4.5%.

The addition of physiologic saline to blood resulted in consistently high cell volumes when the blood was centrifuged for 5 minutes at 1800 r.p.m. The mean deviation was 4.2% when compared with the 60-minute control, and the maximum deviation was 6%.

When unaltered heparinized blood was subjected to consecutive 5-minute centrifugations for 1 hour at 1800 r.p.m. (Fig. 2) the cell volume reading at the end of the first 5 minutes was 60.8% and then fell to 50.8, 49.5, 48.5, and 47.5% at 10, 15, 20 and 25 minutes respec-

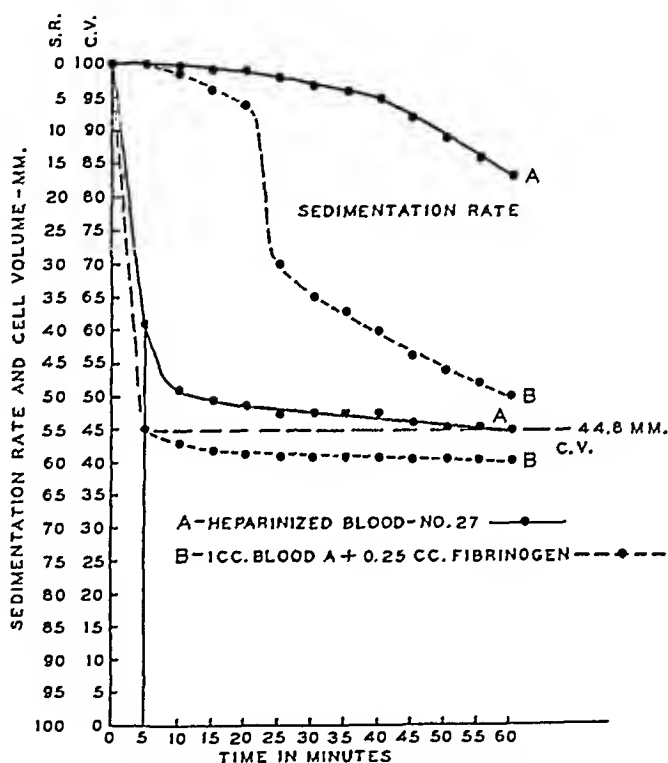


FIG. 2.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

tively until complete packing occurred at 60 minutes and a constant reading of 44.8% was obtained. When 1 cc. of the same blood containing an added 0.25 cc. of fibrinogen solution was centrifuged for 5 minutes at 1800 r.p.m. the cell volume was 45%, almost identical with the cell volume determination of 44.8% observed in the unaltered blood centrifuged for 60 minutes (Fig. 2). During the subsequent 5-minute periods of centrifugation the cell volume fell to 43, 42, 41.2 and 40.9% at 10, 15, 20 and 25 minutes respectively as a result of dilution with the fibrinogen solution. The sedimentation rate was increased from 17 to 50 mm. at the end of 1 hour by the addition of 0.25 cc. of the fibrinogen solution to 1 cc. of blood (Fig. 2).

Figure 3 demonstrates the effect of fibrinogen upon blood with a

slow sedimentation rate. The addition of 0.25 cc. of the fibrinogen solution to 1 cc. of this blood increased the sedimentation rate from 5 to 49 mm. in 1 hour.

After 5 minutes of centrifugation at 1800 r.p.m. the cell volume reading of the control blood specimen was 67% compared with a determination of 48% for the same blood containing the added fibrinogen centrifuged for the same period of time at the same speed. At the end of 60 minutes of centrifugation, however, the control cell volume was 48%, identical with the 5-minute determination of the blood containing added fibrinogen (Fig. 3).

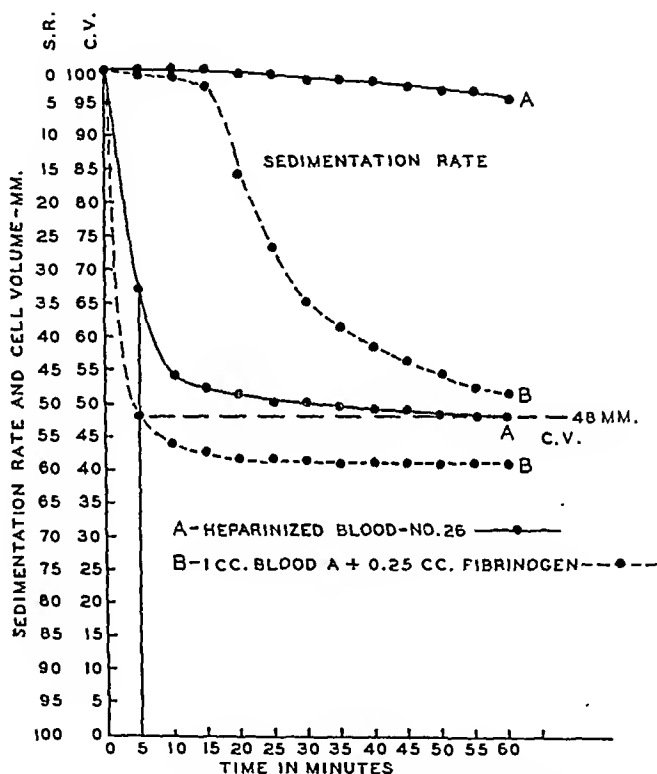


FIG. 3.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

The 5-minute method of determining cell volume is equally accurate for blood with a high cell volume and slow sedimentation rate as demonstrated in Figure 4. Although the sedimentation rate of the blood was increased from the original rate of 0.5 mm. to only 5 mm. in 1 hour by the addition of fibrinogen, the 5-minute centrifugation of this blood yielded a cell volume of 67%, identical with the cell volume obtained by centrifuging the control blood for 60 minutes at 1800 r.p.m. The cell volume reading of the control blood after 5 minutes of centrifugation was 86.5% (Fig. 4). Centrifugation of the unaltered blood for 45 minutes at 3000 r.p.m. also yielded a cell volume reading of 67%, again confirming the accuracy of the 5-minute determination.

The accuracy of the method is not affected by low cell volumes or

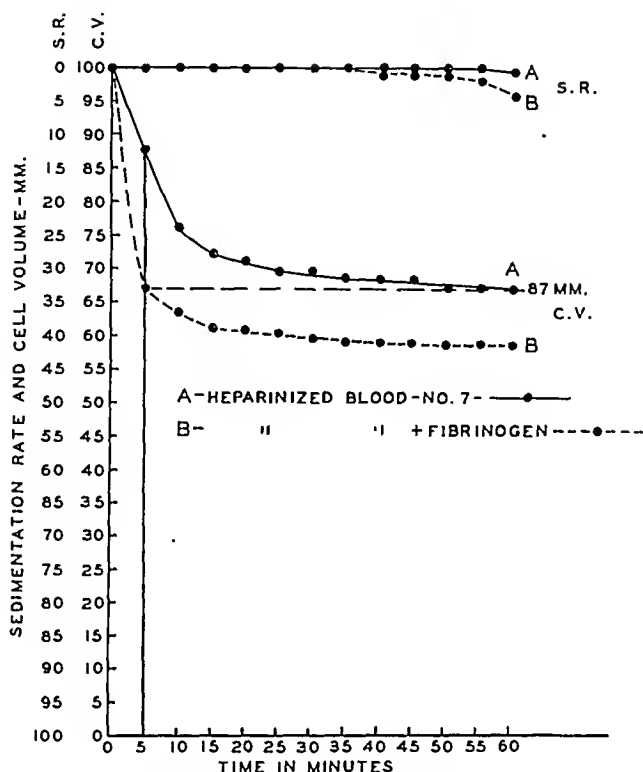


FIG. 4.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

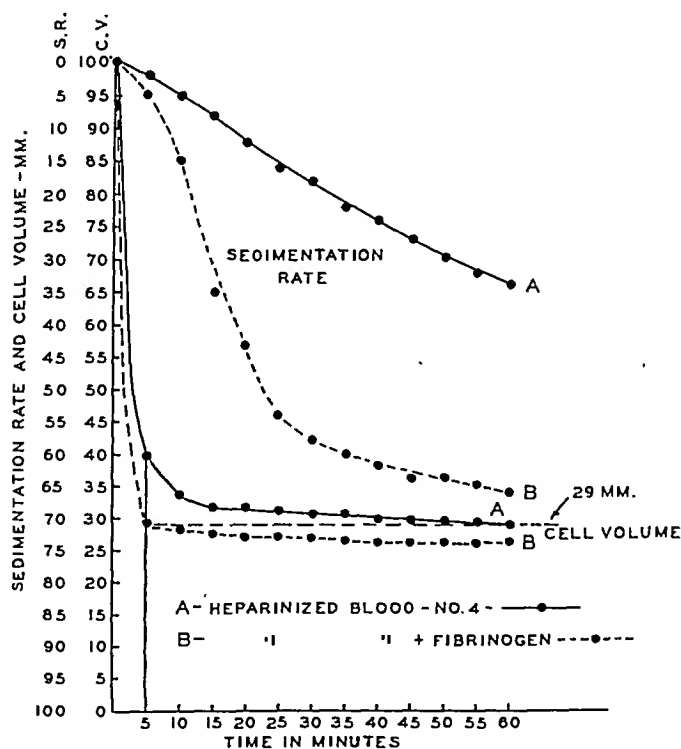


FIG. 5.—The effect of fibrinogen upon the sedimentation rate and cell volume of blood.

high sedimentation rates. This is demonstrated in Figure 5. The cell volume of the blood containing added fibrinogen was 29% after 5 minutes of centrifugation at 1800 r.p.m. The unaltered blood yielded cell volume readings of 39.8% after 5 minutes of centrifugation and 29% after 60 minutes of centrifugation, the latter identical to the 5-minute cell volume of the blood containing the added fibrinogen. The sedimentation rate of the control blood was 34 mm. in 1 hour and was increased to 66 mm. by the addition of the fibrinogen solution.

**Discussion.** There are two factors responsible for this rapid method of determining cell volume. The primary factor is the fibrinogen itself. The addition of an excess of fibrinogen to blood increases the size of the red cell aggregates thus promoting very rapid settling so that the cell volume is reached within 5 minutes of centrifugation at 1500 to 1800 r.p.m.

The second factor is that of dilution. The addition of 0.004 gm. fibrinogen in the dry salt form to 1 cc. of blood does not result in as accurate and consistent cell volume determinations after 5 minutes of centrifugation as does the addition of 0.25 cc. of the fibrinogen solution containing 0.004 gm. fibrinogen as recommended. It has been repeatedly demonstrated that the rate of settling of blood cells is increased by dilution. Cutler<sup>2</sup> believes that the dilution factor increases the rate at which the red blood cells fall because the "activating factors in the plasma," presumably fibrinogen, "have a greater opportunity to drive the remaining cells into rouleaux." Others<sup>8</sup> believe that by decreasing the number of particles in suspension the mean free path of such particles becomes greater, and the rate at which the particles settle is increased.

That the dilution factor alone is not responsible for this method of determining cell volume is shown in Table 3. The addition of 0.25 cc. of homologous plasma to 1 cc. of blood yielded inaccurate and variable cell volume determinations after 5 minutes of centrifugation. This may be explained, in part, by the variation in fibrinogen content of different blood plasmas. Grossly inaccurate and high cell volume determinations were noted after a similar dilution of the blood with physiologic saline since saline inhibits the aggregation of red blood cells.

If heparinized blood is subjected to consecutive 5-minute centrifugations for 60 minutes at 1800 r.p.m. and the level of the red blood cells is recorded graphically, the resulting figure is a vertical curve consisting of two phases (Figs. 2, 3, 4 and 5). The first phase is that in which the red blood cells descend very rapidly in the tube as a result of centrifugation; this phase is of 10 minutes' duration and is graphically represented by a straight line which may be vertical or slightly diagonal, depending upon the size of the cell aggregates. The second phase is that of packing and is influenced by the degree of anemia or dilution. This phase is complete when the level of red blood cells remains constant after several consecutive 5-minute periods of centrifugation. At 1800 r.p.m. this phase requires 40 to 50 minutes of centrifugation. At this point the cell volume is attained.

When the fibrinogen solution is added to the blood rapid aggregation

of the red blood cells occurs and large cell aggregates are formed. Since the rate of settling depends upon the size of the cell aggregates the red blood cells fall very rapidly upon centrifugation at 1500 to 1800 r.p.m. The curve becomes more vertical and is shifted to the left so that the first phase is complete in 5 minutes (Figs. 2, 3, 4 and 5). This period of rapid settling determines the cell volume of the blood. The cells have fallen so rapidly that the cell volume determination after 5 minutes of centrifugation is the same as that of the unaltered blood after 60 minutes. Centrifugation of this blood containing the added fibrinogen solution for more than 5 minutes produces the onset of the second phase. Since this second phase is influenced considerably by the degree of dilution further packing of the cells occurs, and the cell volume falls below that of the unaltered blood (Figs. 2, 3, 4 and 5). For this reason it is very important to time the 5-minute period of centrifugation accurately. If this is not done the cell volume determinations will be low as a result of the dilution factor. The use of an interval timer is the simplest means of insuring accuracy.

The phases of rapid settling and packing observed in blood subjected to consecutive 5-minute periods of centrifugation are very similar to the phases of sedimentation and packing described by Cutler<sup>2</sup> for rapidly sedimenting blood.

The pronounced accelerating effect of the fibrinogen solution upon the sedimentation rate of erythrocytes is demonstrated in Table 2 where increases in the sedimentation rate of 300% or more may be observed. If the sedimentation rates are recorded graphically vertical curves "indicating the most rapid form of sedimentation"<sup>2</sup> are observed when the fibrinogen solution is added to blood (Figs. 2, 3 and 5). All sedimentation rates in this communication are recorded directly without correcting for anemia or dilution since we are concerned primarily with the sedimentation rate of the blood containing the added fibrinogen solution in its relation to the rapid determination of cell volume rather than with the corrected sedimentation rate as a manifestation of disease. Although there is considerable controversy concerning the advisability of correcting for anemia, the addition of 0.25 cc. of homologous plasma to 1 cc. of blood does result in a moderate increase in the sedimentation rate, but the increase in rate does not approach that caused by the addition of 0.25 cc. of the fibrinogen solution to an equal amount of blood. Physiologic saline decreases the sedimentation rate. Neither homologous plasma nor saline is satisfactory for the rapid method of determining cell volume.

It should be emphasized that the time and dilution factors described have been standardized for the Wintrobe hematocrit and that this rapid method is accurate only when the Wintrobe hematocrit is used. The above principles, however, may be applied to any other hematocrit.

The average cell volume determination by the 5-minute method for 10 healthy men and women was 45.9 and 41.5% respectively, comparing favorably with the normal values of other investigators.<sup>6,7,10</sup>

The advantages of a rapid and accurate method of determining cell volume in the study of shock, hemorrhage and dehydration are obvious. The method of determining the volume of packed red cells in 5 minutes

as described is simple, does not require a high speed centrifuge and is as accurate as the present methods requiring 30 to 90 minutes of centrifugation.

**Summary.** 1. Centrifugation for 5 minutes of heparinized blood containing an electrophoretically pure bovine fibrinogen solution yielded cell volume determinations which were accurate to within 0.5% of the cell volumes of unaltered blood centrifuged for 60 minutes at 1800 r.p.m. The accuracy of the 5-minute method was within the range of accuracy of present methods requiring 30 to 90 minutes of centrifugation.

2. This rapid method of determining cell volume was applied to 50 patients and was found exceedingly accurate within a wide range of cell volumes (17 to 68%) and sedimentation rates (0.5 to 42 mm.). The mean deviation was 0.24% and the accuracy of the method was not affected by the range of cell volume or sedimentation rate.

3. Comparative 5-minute cell volume determinations of blood containing added fibrinogen, homologous plasma and saline indicated that accurate and consistent results were obtained only in the blood specimens containing the added fibrinogen solution.

4. The most accurate results were obtained by centrifugation of the blood containing the fibrinogen solution for 5 minutes at 1500 to 1800 r.p.m.

5. When heparinized blood was subjected to consecutive 5-minute periods of centrifugation and the level of the red blood cells was recorded graphically the resulting figure was a vertical curve consisting of two phases: rapid settling and packing. The addition of the fibrinogen solution to blood increased the rate of settling of the erythrocytes and produced a more vertical curve so that the first phase was completed in 5 minutes and recorded the cell volume.

6. The pronounced accelerating effect of the fibrinogen solution upon the sedimentation rate of erythrocytes and the mechanism of the rapid method of determining cell volume were discussed.

This work was aided in part by a grant from the Otho S. A. Sprague Memorial Institute, University of Chicago.

The author wishes to thank both Dr. Edwin J. Cohn and the Armour Laboratories for the purified fractions used in this study. Prepared by the methods developed at the Department of Physical Chemistry, Harvard Medical School, they were supplied by the Armour Laboratories, Chicago, Ill.

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## THE INHALATORY ROUTE FOR PROPHYLAXIS AND TREATMENT OF EXPERIMENTAL INFLUENZA\*

### I. THE DISTRIBUTION OF INHALED MATERIAL

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THE work of Smorodintseff and co-workers<sup>30,31</sup> and Nachaev<sup>18</sup> on the prevention and treatment of human influenza by inhalation of immune horse serum has stimulated our interest in this subject. Complete data on the apparatus and methods used by the Russian authors are not given in their available publications.

The chief route of transmission of epidemic influenza is by direct inhalation of infective material either in the form of small droplets or droplet-nuclei of the Flügge type. Once infection has established itself it is probable that the whole respiratory tract from nasal epithelium to the distal alveoli of the lungs is involved. Nevertheless, uncomplicated influenza must still be regarded as a localized infection, since it is rare that other body sites are infected.

The approach of Smorodintseff and Nachaev in attempting to reach the susceptible or infected area with serum by the only direct route is a most logical one. Intravenous and intramuscular injections of immune serum were attempted during the epidemic of 1918-19 with doubtful results, while it has been repeatedly shown<sup>13,32,39</sup> that the intranasal administration of immune serum to mice is superior to subcutaneous, intra-abdominal or intravenous routes for protection and treatment of the experimental animal.

Before attempting clinical trials of the inhalation method it was decided to investigate the efficiency of the mode of administration in penetrating the deeper passages of the respiratory tract. There is no doubt that intranasal insufflation of fluids results in an uneven distribution of the material. The ideal method of administration to humans would be one which not only would distribute the inoculated material evenly but would reach the deeper respiratory passages as well. The

\* The opinions advanced in this paper are those of the writers and do not represent the official views of the Navy Department.

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disadvantages of the intranasal route in mouse inoculations of both serum and virus suspensions led Zellat and Henle<sup>39</sup> to suggest atomization of serum in order to assure its more uniform distribution.

It is evident that any device to be used in inhalation experiments must be capable of producing a fog of very finely divided particles and, at the same time, operate at a high rate of efficiency in terms of amount of liquid atomized per unit volume of air required for the atomization process. In this paper, the first part of a series, is described the construction and preliminary performance data of an improved atomizer developed in this laboratory.

All atomizers operate on essentially the same principle, namely, impinging air and liquid at sufficient velocity to break up the liquid into small particles. The ordinary household fly-sprayer is a good example of a simple atomizer employing a relatively slow moving stream of air directed at right angles across a liquid source rising in a capillary tube. Several refinements have been made in this basic type atomizer. A modified Wells atomizer<sup>38</sup> built into a Florence flask and employing a mixture of liquid and air driven at high velocity through a small orifice has given good results. Such atomizers have the advantage of delivering a uniformly small particle size by screening out the larger particles within the body of the flask. These droplets are returned to the reservoir and then re-atomized. This type of atomizer has the disadvantage of a very low total output per unit time, and, for work of the nature being done in this laboratory, is quite unsuitable.

The atomizer herein described delivers uniformly small particles in an efficient manner at the rate of approximately 55 to 65 cc. per hour and yet operates within a pressure range readily obtainable in the laboratory. Figure 1 shows the atomizer built for these studies; it is a composite modification of the existing atomizers of this type rather than something entirely new.

The body of the atomizer is a 2 liter Florence flask. The jet, *A*, is made of  $\frac{1}{4}$  inch (I.D.) glass tubing drawn to a tip with an orifice of approximately  $\frac{1}{16}$  inch. Within this tube is fused a smaller tube of  $\frac{1}{16}$  inch (I.D.) drawn to a tip of approximately  $\frac{1}{32}$  inch orifice. The air stream passes up the inner tube and out the common orifice of the two tubes, thereby creating a partial vacuum in the space between the adjacent walls of the inner and outer tubes. The small hole shown at the base of jet *A* leads to the reservoir of liquid which is drawn up into this space and is driven out into the flask with the air stream from the inner tube. The mixture of liquid and air is then met by the opposing air streams from jets *B* and *B'*. These two jets with orifices of approximately  $\frac{1}{32}$  inch, are made of  $\frac{1}{8}$  inch glass tubing and are joined together at the base. The placement of the two jets for producing best results was determined empirically. When the air jets are placed very close to the air-liquid jet the delivery of liquid is almost stopped; on the other hand, if they are fixed beyond the optimal distance much of the air-liquid mixture is by-passed around the opposing air streams and the additional effect of finer dispersion of the liquid particles is lost. A considerable portion of the atomized liquid



impinges on the surface of the Florence flask, the larger droplets returning to the reservoir while the small particles are carried out of the atomizer with the air current.

The operating efficiency of the atomizer is influenced by the dimensions of the air jets and the air-liquid jet and by the air pressure employed. Other factors, such as temperature and relative humidity of the air, the viscosity, boiling point, temperature, etc., of the liquid will also influence its performance. The occurrence of a distillation effect, inversely related to the humidity of the air introduced, has been noted. These problems are being investigated in our laboratories at this time. The following calibrations are tentative and have purposely

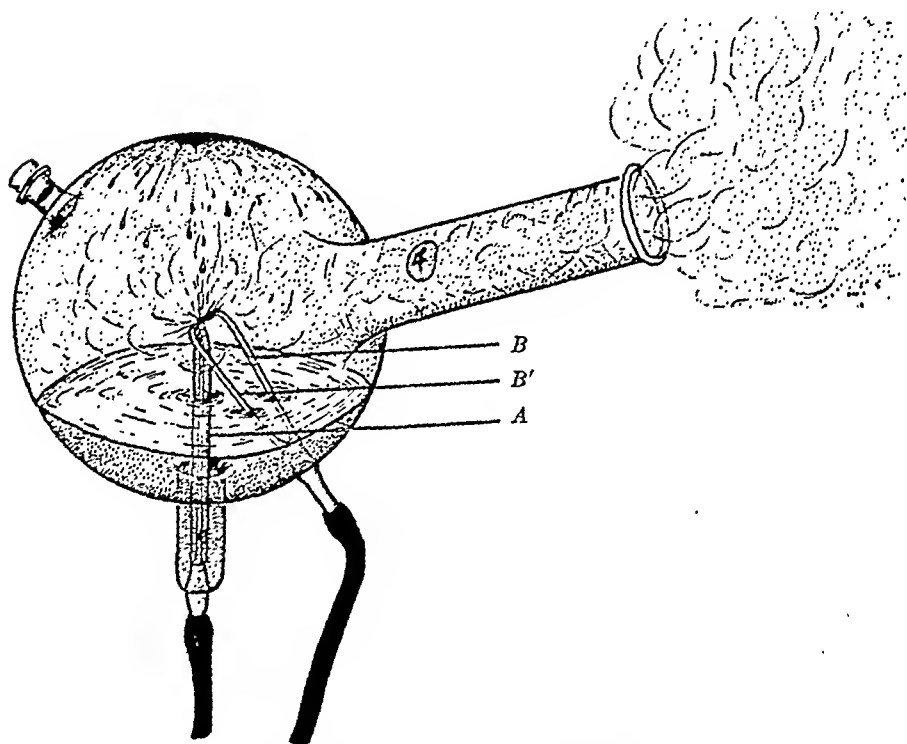


FIG. 1.—Atomizer which delivers uniformly small particles at rate of approximately 55 to 65 cc. per hour at pressure range readily obtainable in the laboratory.

ignored many of the complicating factors in order that rough performance data could be obtained and utilized for investigating direct immunization of the respiratory tree.

The curve shown in Figure 2 depicts the efficiency data for one of these atomizers at various air pressures. The calibrations were made at room temperature (21° C., relative humidity averaging 55%) using distilled water as the liquid. The air pressure is shown on the abscissa in terms of centimeters of mercury while the volume of water atomized per unit volume of air (cc./liter) is plotted on the ordinate. It will be seen that within the ranges of pressure from 20 to 55 cm. of mercury the calculated efficiency is expressed in a smooth curve. The efficiency

of the atomizer increases with each increment in operating pressure, but at a gradually diminishing rate. At 40 cm. of mercury the atomizer has an output of approximately 0.024 cc./liter of air delivered to the atmosphere. The upper pressure ranges were used most commonly because the greater output of the atomizer permits more rapid attainment of equilibrium in the experimental chamber.

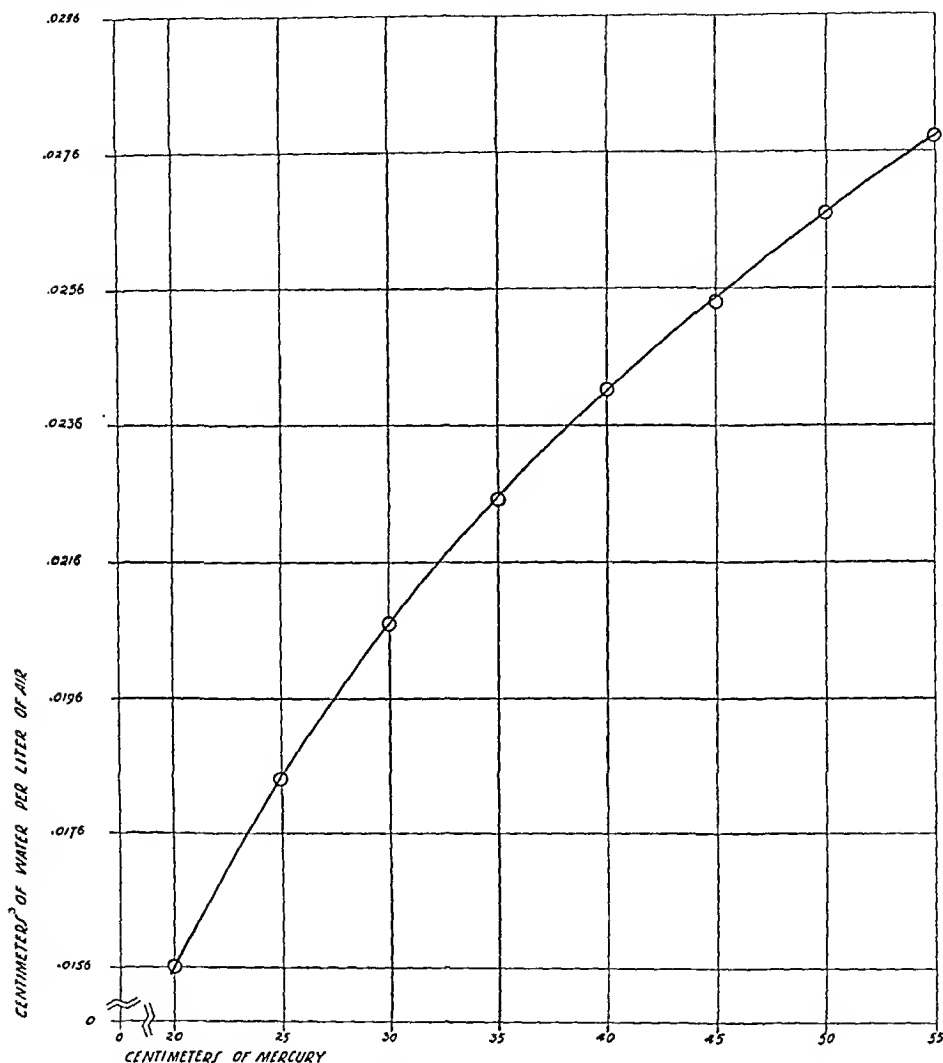


FIG. 2.—Efficiency curve—centimeters<sup>3</sup> of water delivered per liters of air.

It has not been deemed necessary to determine accurately the particle size delivered by the atomizer at this time. Although several methods are described in the literature we have not found one which we feel would be sufficiently accurate to justify such determination.

In order to investigate the extent to which a suspension atomized through the apparatus described would penetrate into the lung substance, a full grown *Macacus rhesus* monkey was confined in an atmosphere of India ink sprayed in a 1:10 dilution for 1 hour. The air

pressure was held at 40 cm. of mercury. Upon completion of the atomization the animal was chloroformed and autopsied immediately to determine the carbon distribution.

No carbon was observed in the mouth, thus suggesting that most of the inspired vapor had been taken in through the nose. The vestibules of the nares and the nasal cavities in general contained carbon, although the distribution was not uniform, more of the particles collecting in the anterior and posterior thirds. Carbon particles were found entangled in the mucus covering the membrane of the lateral and septal walls as well as on the conchæ. Considerable quantities of the ink had collected in the nasopharynx and some appeared in the openings of the auditory tubes.

The oral pharynx had apparently been cleared of any grossly detectable amount of carbon through swallowing of the salivary and oral secretions. This was also true of the esophagus, which showed negligible traces of carbon, but the presence of a considerable amount of ink in the stomach indicated that the particles collecting on the pharyngeal surface, either from the nasal or oral cavities, had been rapidly swallowed. Some of the carbon which had not been caught in the mucus of the nasal passages found a partial barrier in the vocal cords, which were stained black. Some carbon was also observed in the ventricular recess.

Carbon was found in a spotty distribution along the trachea and bronchi. Wherever the inspired air current had turned at an angle due to the branching of the respiratory tract, more carbon was seen to have accumulated. Examination of slices of the fixed lung tissue under the dissecting microscope showed that the inspired material had penetrated into the alveolar spaces and was readily detected immediately below the visceral pleura. These observations were confirmed by examination of stained sections. The latter also revealed "dust cell" aggregates, an essentially normal feature resulting from the chronic inhalation of dust particles and usually seen in most monkeys of this age.

In order to compare the distribution of particulate material obtained by spraying with that obtained by intranasal instillation, 4 cc. of a 1:10 aqueous dilution of India ink was administered over a period of about 5 minutes by dropper instillation into the nostrils of a full-grown *M. rhesus* monkey maintained under light ether anesthesia. The animal was then killed by injecting 2 cc. of ether intracardially and an autopsy performed. The lungs were removed in continuity with the bronchi, trachea and larynx, and the air passages from the epiglottis to the smallest bronchi were then laid open with the scissors. Considerable carbon was found entangled in mucus in the larynx, especially above the vocal cords. The visible distribution of carbon in the trachea was irregular, a few black patches of carbon-containing mucus being found at intervals along the tract. Occasional minute deposits of ink could be detected within the smaller bronchi, especially in the middle and lower lobes. There were in all not more than 10 areas of diffuse blackening in the respiratory tissue proper. How much carbon

had reached the alveoli was not ascertained, but it was quite evident that it was very small compared with the amount seen in the lungs of the animal which had been sprayed.

No carbon could be detected on the pharyngeal mucous membrane, nor in the upper half of the esophagus. The lower half of the esophagus contained an abundance of the carbon-mucus mixture and in the stomach there was estimated to be more than half of the administered ink.

A considerable amount of carbon embedded in mucus was found in the nasopharynx, and the posterior third of the nasal passageway was also congested with black mucus. This was likewise true of the vestibule and the anterior part of the nasal cavity proper. The mucous membrane covering the middle third of the turbinates, septum, and lateral walls was relatively free of carbon. Judging from the amount of carbon found to have been swallowed within a few minutes after introduction into the nares, the bulk of the carbon-mucus mixture still remaining in the nasal cavity would also have found its way into the alimentary tract in a short time. The carbon-free zone of oral pharynx and upper esophagus points to the operation of a rapid and very effective swallowing mechanism for clearing the particulate matter that becomes grounded in the nasal mucus and is moved along by ciliary action to the pharynx.

One inescapable conclusion is that the intranasal instillation method provides a rather direct route to the alimentary tract, especially when fluid is introduced that may act to incite an increased flow of mucus. The inhalation of a finely vaporized spray directly into the larynx through the mouth should permit much less swallowing of the material.

In order to determine roughly the proportionate distribution of inspired particulate matter, a third experiment was carried out using a suspension of radio-active chromic phosphate ( $\text{Cr}_2\text{PO}_4$ )\* in place of the India ink.

The tidal air of a *M. rhesus monkey* (weight,  $7\frac{3}{4}$  pounds) under intravenous nembutal anesthesia was determined by means of an intratracheal cannula connected to a respirometer. Several determinations were made and the consecutive results are presented in Table 1.

TABLE 1.—TIDAL AIR DETERMINATION (*M. rhesus*).

Time of measurement (min.)	Average respirations per min.	State of breathing	Air per minute (cc.)
18.0 . . . . .	32	Shallow	553
3 6 . . . . .	26	Deeper; regaining consciousness	905
(More nembutal administered)			
2.4 . . . . .	30	Deep; partially conscious	769
18 0 . . . . .	26	Shallow	543
13 6 . . . . .	25	Shallow	542

It is evident that if the animal is kept at a constant level of anesthesia its tidal air can be determined and close checks obtained. The

\* Obtained through the courtesy of Dr. Hardin Jones, Donner Medical-Physics Laboratory, University of California, Berkeley, Calif

average for the three determinations (1, 4 and 5) is 546 cc. per minute. As soon as the animal regains partial consciousness the tidal air increases markedly. It was not possible to obtain figures for tidal air of the fully conscious monkey.

The next day the same monkey was put under intravenous nembutal anesthesia and placed in a chamber which had previously been subjected to a spray of radio-active chromic phosphate for 37 minutes to bring the concentration of the fog to equilibrium. The animal was exposed to the spray for 40 minutes. During the 77 minute total spraying 79 cc. of the material, containing 0.1 microcurie per cc., were atomized at a pressure of 40 cm. mercury. At the termination of this period the monkey was killed and autopsied.

Except for a region extending about 4 mm. within the nasal vestibule, where a yellowish-gray deposit was observed, no chromic phosphate could be detected grossly throughout the body of the animal. There were no signs of inflammation or hyperemia in the respiratory or alimentary organs.

A small section from the apical region of the lung was frozen and dried *in vacuo* and a free-hand razor blade section weighing 5 mg. made. This was laid out on a piece of blotting paper covered with cellophane and tested against a Roentgen ray film for beta ray emanations. After a 3 day exposure a picture was obtained, indicating that finely atomized particulate matter was uniformly distributed throughout the lungs, the image of the print following the exact outline of the tissue section tested.

The lungs, dissected free from adjoining tissue and separated from the trachea, weighed 24 gm. They were frozen and dried *in vacuo* and then incinerated in a flat porcelain dish at a temperature which left a residue of ash including the chromic phosphate. This ash was analyzed for radio-activity by means of a beta-counter, and the value obtained used to determine the total amount of atomized chromic phosphate remaining in the lungs after inhalation.

Beta counts made on the chromic phosphate residue remaining in the atomizer after atomization showed an increase of 100% from 0.1 to 0.2 microcurie per cc., indicating that a distillation effect was taking place during the spraying. By calculation of the amount of radio-activity contained in the 97 cc. of the original sample introduced into the atomizer and the amount remaining in the residue, it was possible to determine the average activity of the 79 cc. which were delivered to the atmosphere of the monkey. From the known figures on air and fluid delivery of the atomizer and the previously determined figures on tidal air under nembutal anesthesia it was calculated that the monkey had inspired a total of 0.59 cc. of radio-active material. By radio-activity measurements of the lungs of the same monkey it was calculated, by comparison with a control chromic phosphate sample, that approximately 0.1 cc. of the material or 0.01 microcurie had been retained in the lungs. In view of the fact that a second monkey treated similarly showed that of the total amount of radio-active material contained in the body, 75% was in the respiratory tract and only 25%

in the alimentary tract, the difference between the amount calculated to have been inspired, 0.59 cc. and the amount retained, 0.1 cc., must be considered to be due chiefly to the material exhaled. Heubner<sup>14</sup> states that between 50% and 80% of an inhaled suspension is exhaled by man. With an undeterminable variable of such magnitude at play it is difficult to assess the accuracy of our figures until repeated confirmations are obtained. Theoretically, using a radio-active substance as an indicator would seem to be a relatively accurate experimental method to determine such values for animals.

Another radio-autographic print was made with a teased lung section from one of a group of mice exposed to radio-active chromic phosphate for 1 hour, and showed the same penetration and uniform distribution of atomized particles in the lung tissue proper.

The data summarized above offer such clear-cut evidence of the superiority of the inhalatory method that it was considered advisable to employ it in experiments designed to test the value of influenzal antiserum for the protection and treatment of man.

**Summary.** 1. An atomizer has been described which is capable of producing a fine particle mist with high efficiency in terms of volume of atomized liquid per unit volume of air delivered. Preliminary performance data are presented.

2. India ink and radio-active chromic phosphate were used as indicators in experiments on monkeys and mice to compare the inhalatory method and the intranasal route. The superiority of the inhalatory method with respect to distribution and penetration of both materials was definitely established.

## II. IMMUNE SERUM IN PROPHYLAXIS AND TREATMENT\*

BY THE PERSONNEL OF NAVAL LABORATORY RESEARCH UNIT No. 1

**Introduction.**—The application of immune serum directly to the respiratory tract for the purpose of conferring a passive immunity is not a recent innovation. Besredka<sup>2</sup> as long ago as 1920 advocated intratracheal injections of immune serums for development of a general passive immunity. He claimed that this method would not only confer a strong, immediate immunity, but would obviate the danger of anaphylactic shock in those cases which would be susceptible to the usual intravenous serum administration. However, he failed to compare the resultant immunity from intratracheal inoculation with that produced by equal amounts of serum administered intravenously, or intramuscularly. Jones<sup>15,16</sup> demonstrated that the intratracheal route does not compare favorably with intraperitoneal inoculations in ability to confer a general passive immunity.

Fox,<sup>9</sup> in a further investigation of the problem, confirmed and extended the observations of Jones that, although intratracheal administration of antiserum produces a definite blood antibody level, it is "a

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grossly inefficient way to induce a general passive immunity" when compared with the intravenous or subcutaneous routes. In the course of these experiments it was demonstrated that antibodies appear in the blood stream only slowly and in low titer, and, conversely, that these antibodies are retained in the lungs as long as 5 days. This definite indication of the relative impermeability of the lungs to antibody protein has been further corroborated by the report of Drinker, Warren and MacLanahan<sup>7</sup> whose work demonstrated that proteins such as horse serum and crystallized hemoglobin were absorbed but slowly from the lungs and, contrary to expectations, invariably appeared first in the blood rather than in the lymphatics.

Fox<sup>9</sup> has quite clearly stated the case for the respiratory route of administration of immune serums. He writes: "In pulmonary diseases amenable to serum therapy certain benefits accruing to local use of serum are suggested. In addition to the possible reduction of the peril of anaphylaxis as suggested by Besredka, one may enumerate particularly (1) the much higher local antibody content of the lung obtainable after local administration of small amounts of serum than follow injection of much larger amounts intravenously, and (2) the prolonged retention of such antibody."

To turn to our problem of influenza, we may concern ourselves with the arguments for and against the use of immune sera administered locally for the prevention and treatment of this disease. The most obvious advantage of such a method would be the immediate, albeit fleeting, production of an immune state, thus supplying a rapid means of combating an epidemic in a susceptible group. Were successful vaccination against influenza a *fait accompli*, enabling us to minimize significantly the susceptibility of our almost universally susceptible population, we might regard serum prophylaxis as unwarranted, but at the present time the status of influenza vaccines is not too encouraging. The reports of Russian workers<sup>18,30,31</sup> employing horse serum both prophylactically and therapeutically indicate the value of the treatment while little mention is made of contraindications or untoward reactions.

Theoretically, however, there would seem to be some danger involved in the repeated inhalation of a heterologous serum. It is highly probable that sensitization to the foreign proteins of the serum would result in man, if we can consider the reaction to be comparable to that of the guinea pig even in a qualitative manner. Sensitization and fatal shock by inhalation techniques<sup>1,4,11,26,28,29,33</sup> have been repeatedly observed in this animal. To minimize the risk of allergic response in man, the ideal serum for use as an inhalatory immunizing agent would be human convalescent or hyperimmune serum. However, it is difficult to obtain large quantities of this material in sufficiently high titers. Rabbit serum might be less likely to cause sensitivity reactions than horse serum, but here again for the quantities of serum needed in any large scale operations the rabbit is not as satisfactory as the horse. Nevertheless, this fact may be outweighed by the higher immune titers that can be produced in the rabbit.

The use of a salted-out globulin fraction of horse serum, although enabling a significant concentration of antibodies to be made, does not eliminate the sensitivity problem. Recently developed methods of purification and despeciation of immune sera by enzyme digestion would seem to have application here.<sup>6,17,20,23,24,25,36</sup> Such methods of purification alter the specificity of the serum proteins to such an extent that cross-sensitivity reactions to the original serum are greatly reduced. At the same time the digestion process brings about an actual reduction in antigenicity of the proteins involved, as demonstrated by large quantitative differences in sensitizing and reacting properties. The inevitable drop in antibody content of the despeciated serum can be tolerated providing subsequent concentration would raise the product to an effective potency.

In view of the facts that the absorption of proteins from the lung into the blood is a slow process and the bronchial tree of man is not heavily coated with smooth muscle as in the guinea pig, we should not expect to see acute shock resulting from inspiration of serum or serum fractions into the respiratory tree. There is, however, a definite possibility of delayed reactions of asthmatic nature appearing within a few hours time in hypersensitive individuals. Administration of material such as serum proteins by inhalation would be contraindicated in acknowledged asthmatics, while the number of reactions in others could very likely be greatly reduced by a thorough screening, *i. e.*, detection of serum-sensitive individuals by accurate history-taking combined with skin and conjunctival tests. Positive reactors conceivably may be desensitized by intradermal administration of serum or by the inhalation of successively larger amounts.

The danger of sensitization and subsequent production of reactions in a group of individuals subjected to treatment during an epidemic is decidedly reduced merely by the necessary timing of the inhalations. Since the passive immunity conferred by the serum is effective only for a relatively brief period it would probably require one, two, or possibly even three treatments per week to maintain an individual in the immune state. With such spacing of antigenic stimuli it is improbable that reactions would occur during a few weeks treatment, since each inhalation would serve to induce temporary desensitization and insufficient time would elapse between inhalations for the production of a true hypersensitive state. If it is practicable to employ a despeciated, purified product instead of whole serum or its globulin fraction, some of these speculative problems may be avoided. This aspect of the problem is being investigated at the present time.

In anticipation of clinical trials of immune serum or a fraction thereof to be administered by inhalation, certain laboratory experiments were undertaken to confirm and extend observations on the value of the method in prophylaxis and treatment.

**Preparation of Horse Immune Plasma and Its Globulin Fraction.** Horses were immunized by weekly subcutaneous inoculations of active mouse lung viruses. Sample bleedings were made at intervals and tested by a red blood cell agglutination-inhibition method<sup>21</sup> until a



sufficiently high titer was obtained, the first volume-bleeding being made 9 days after the tenth inoculation. The antigen inoculated consisted of 50 cc. of a mixture of PR-8, W.S., Philadelphia, and Lee strains of virus prepared in sterile broth in a concentration of 10% mouse lung by weight. Three bleedings were made from 1 horse, allowing time for one virus inoculation between the second and third bleedings, and 15 liters of plasma recovered. The presence of the fibrinogen in the plasma proved to be a troublesome factor, so that subsequently all bleedings have been performed to obtain serum.

The plasma was salted out by the sodium sulfate method and the fraction precipitating between 8% and 21% concentration was recovered and redissolved in a volume of 3 liters, or one-fifth of the original plasma volume. (In later fractionations 17.5% sodium sulfate was used as the upper limit, for the fraction between 17.5% and 20% apparently does not contain protective antibodies.) To this concentrate was added 0.3% tricresol as a preservative. This globulin fraction was light greenish in color and of a viscous consistency. Microkjeldahl determinations on the plasma and the globulin gave the following results for total nitrogen: whole plasma, 8.44 mg. nitrogen per cc.; concentrated globulin, 17.20 mg. nitrogen per cc.

Neutralization tests against 1000 MLD of PR-8 mouse lung virus gave the following comparative results, calculated according to the method of Reed and Muench:<sup>27</sup>

	50% mortality end-point	50% maximum score end-point
Whole plasma . . . . .	1:865	1:250
Globulin . . . . .	1:3450	1:1540

These results would indicate that most of the antibody content of the plasma had been recovered in the 8% to 21% globulin fraction, since the titer of the globulin, a five-fold concentrate, is approximately 4 times that of the original plasma by comparison of 50% mortality end-points and 6 times that of the plasma when 50% maximum score end-points are compared.

A neutralization test on the globulin run against 1000 MLD of F-99\* egg virus gave a 50% maximum score end-point of 1:1380, although the viruses used to immunize the horse did not specifically include an F-99 strain. The globulin also exhibited strong protective powers against a Melbourne strain of virus, indicating the wide latitude of its antibody activity within the "A" type group of influenza viruses.

**Protection of Mice by Intranasal Inoculation of Plasma or Inhalation of Globulin.** It has been definitely shown that the administration of influenza immune serum by the intranasal route is more efficient than intra-abdominal or intravenous injection.<sup>13,32,39</sup> Zellat and Henle<sup>39</sup> further proved that mice could be protected against many lethal doses of virus if they first inhaled a serum spray for 2 hours.

It was our desire to confirm some of these results with respect to intranasal inoculation and inhalation. Accordingly, groups of mice

\* Obtained through the courtesy of Dr. Joseph Stokes, Jr.

were sprayed for 30 minutes, 60 minutes, or 120 minutes with a two and one-half-fold dilution of our immune globulin. The 50% mortality titer of this diluted material can be considered as 1:1340. Each mouse was kept in an individual compartment within the spray chamber in order to prevent bunching of the animals during exposure to the globu-

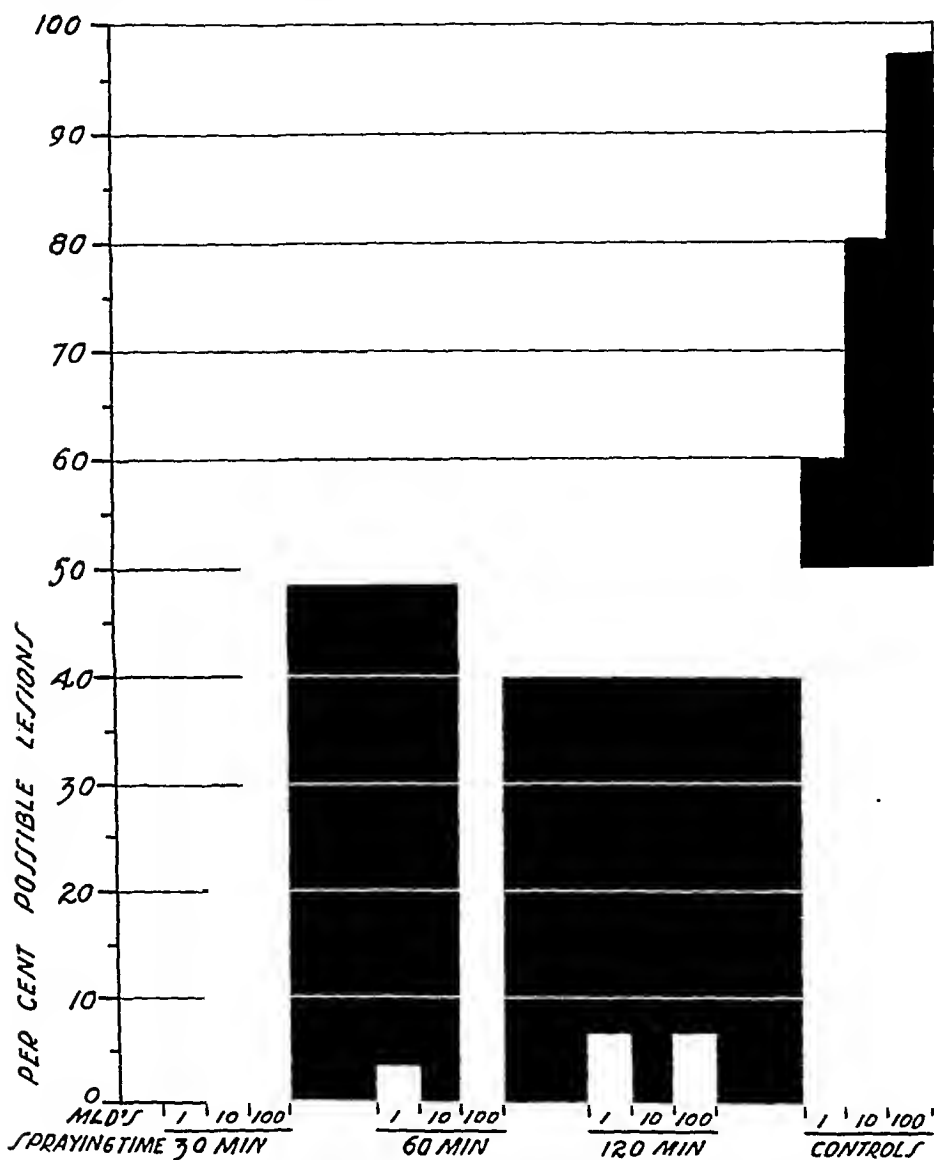


CHART 1.—Protection of mice by inhalation of immune globulin.

lin fog. Four hours after the spraying the mice were inoculated intranasally with graded doses of W.S. mouse lung virus. During a 10 day period of observation the animals succumbing were autopsied; at the end of this time all survivors were sacrificed and their lung lesions scored. Chart 1 is a graphic presentation of the results obtained. The vertical columns represent the per cent of possible lesions observed

in each group of 6 mice; for example, if the lesion score of a group of 6 mice adds up to 15, where death within 10 days is counted as 5 and the degree of lung consolidation in survivors is evaluated from 1 to 4, then the per cent of possible lesions observed would be 15/30 or 50%. It will be seen that the degree of protection increases with the time of inhalation of the globulin.

In order to compare the inhalatory route with intranasal inoculation another group of mice was inoculated intranasally at the same time with 0.05 cc. of dilutions of the concentrated globulin solution from 1:5 to 1:2560 in two-fold steps. By comparing the lesion scores resulting from intranasal inoculation of the same graded doses of W.S. virus 4 hours later it was computed that spraying for 30 minutes was comparable to an intranasal inoculation of 0.05 cc. of a 1:232 antiserum dilution, 60 minutes was equal to a dilution of 1:74, and 120 minutes to a dilution of 1:16.

The inhalation of the globulin did not compare as favorably with the intranasal inoculation as had been expected. It was suspected that a distillation effect taking place during atomization might account for the discrepancy and an experiment was set up to investigate this factor. A low titer immune serum was sprayed for 1 hour and the spray collected in a flask immersed in cellosolve and dry ice. Neutralization tests against 1000 MLD of PR-8 mouse lung virus were run on the original serum, the serum remaining in the atomizer after the spraying, and the serum collected during the spraying. Serum dilutions from 1:8 to 1:512 prepared in two-fold steps were used. Table 2 is a summary of the results.

TABLE 2.—SUMMARY OF RESULTS

	50% maximum score point	Total lesions per group	
		Ratio	Per cent
Original serum . . . .	1:69	97/210	46.2
Atomized serum . . . .	1:60	109/205	53.2
Residual serum . . . .	1-140	73/205	35.6

Measured either by the 50% maximum score end-point or by the per cent of possible lesions in each group, the residual serum showed the highest antibody content and the atomized serum the lowest. Here again is evidence of a significant distillation effect, a factor which must be considered in any estimations of inhaled material. The difference in lesions observed by Wells and Henle<sup>27</sup> between mice sprayed with virus and mice inoculated intranasally with the same calculated amount of virus may very likely be due to the distillation factor. The virus which reached their mice was probably less concentrated than their original material.

Another earlier experiment was designed to compare the duration and degree of protection conferred by a pseudo-globulin fraction of the horse plasma with that induced by whole plasma. The pseudo-globulin fraction was prepared by precipitation with 50% ammonium sulfate and dialysis against distilled water until sulfate-free. After removal of insoluble material by centrifugation, the supernatant was diluted

to the original volume of the plasma and brought to isotonicity by the addition of solid NaCl. Micro-kjeldahl measurements of the total nitrogen of these two preparations gave the following results: whole plasma, 8.44 mg. N/cc.; pseudo-globulin, 0.0837 mg. N/cc. Two groups of mice were inoculated respectively with 0.05 cc. aliquots of the plasma or its pseudo-globulin fraction administered by the intranasal route. At intervals of 24 hours, 72 hours, 6 days and 11 days following this treatment batches of mice from each group were tested for immunity by intranasal inoculation of graded doses of W.S. mouse lung virus.

The results are graphically presented in Chart 2, from which it is seen that the whole plasma was markedly superior to the pseudo-globulin fraction in protective power. By the 6th day following the inoculation of the plasma the passive immunity had all but disappeared, showing significant protection against only 1 MLD. These results are similar to those obtained by Henle, Stokes and Shaw<sup>13</sup> using immune mouse serum intranasally. They were able to demonstrate a slight immunity against 10 MLD as long as 10 days after the intranasal administration of the homologous immune serum.

**Treatment of Infected Mice With Plasma or Globulin.** Although the value of immune serum administered by way of the respiratory tract for the treatment of experimental influenza has been demonstrated by other workers, the question of whether repeated treatments are indicated has not been thoroughly investigated in the laboratory. Hare<sup>12</sup> conducted experiments in which two separate intraperitoneal injections of immune ferret serum were given 24 hours apart to previously infected mice, but direct comparisons with other numbers of treatments were not made.

In order to determine more accurately the value of repeated instillations of immune horse serum the following experiment was conducted.

Mice were inoculated intranasally with 10, 100, or 1000 MLD of PR-8 mouse lung virus. At three different time intervals after infection, 6 hours, 30 hours and 72 hours, serum treatment was begun. Each treatment consisted of an intranasal inoculation of 0.05 cc. of the immune serum. The groups of mice were further divided into those receiving 1, 2, 3, or 4 serum treatments, 24 hours elapsing between successive serum instillations. Control mice were inoculated with normal horse serum, 4 instillations for each control group.

The results of this experiment are shown in Chart 3. Each vertical bar represents 10 mice, except in a few instances where non-specific deaths reduced this number to 8 or 9. The importance of repeated administrations of serum in the treatment of influenzal infection is significantly shown by this experiment. It is especially evident in the groups "30 hours, 10 MLD's," "6 hours, 100 MLD's," and "6 hours, 1000 MLD's." In the first instance there was a reduction of lesions from 74% (1 treatment) to 30% (4 treatments); in the second example, a reduction from 22% to 4%, and in the third, from 49% to 8% of possible lesions. In the groups of mice where the serum treatment was not initiated until 72 hours after the virus inoculation, the infec-

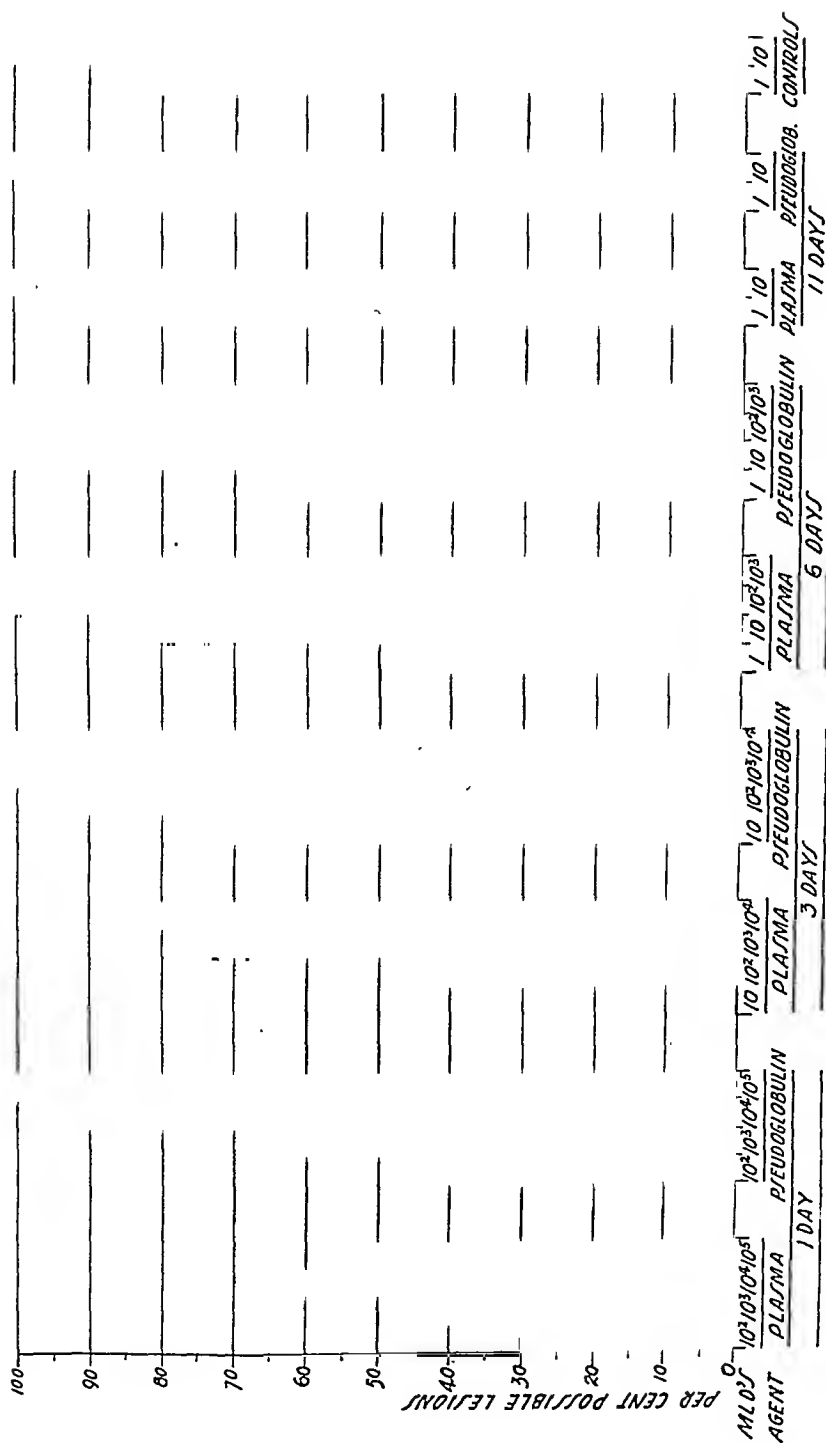


CHART 2.—Protection of mice by intranasal inoculation of immune plasma or pseudoglobulin.

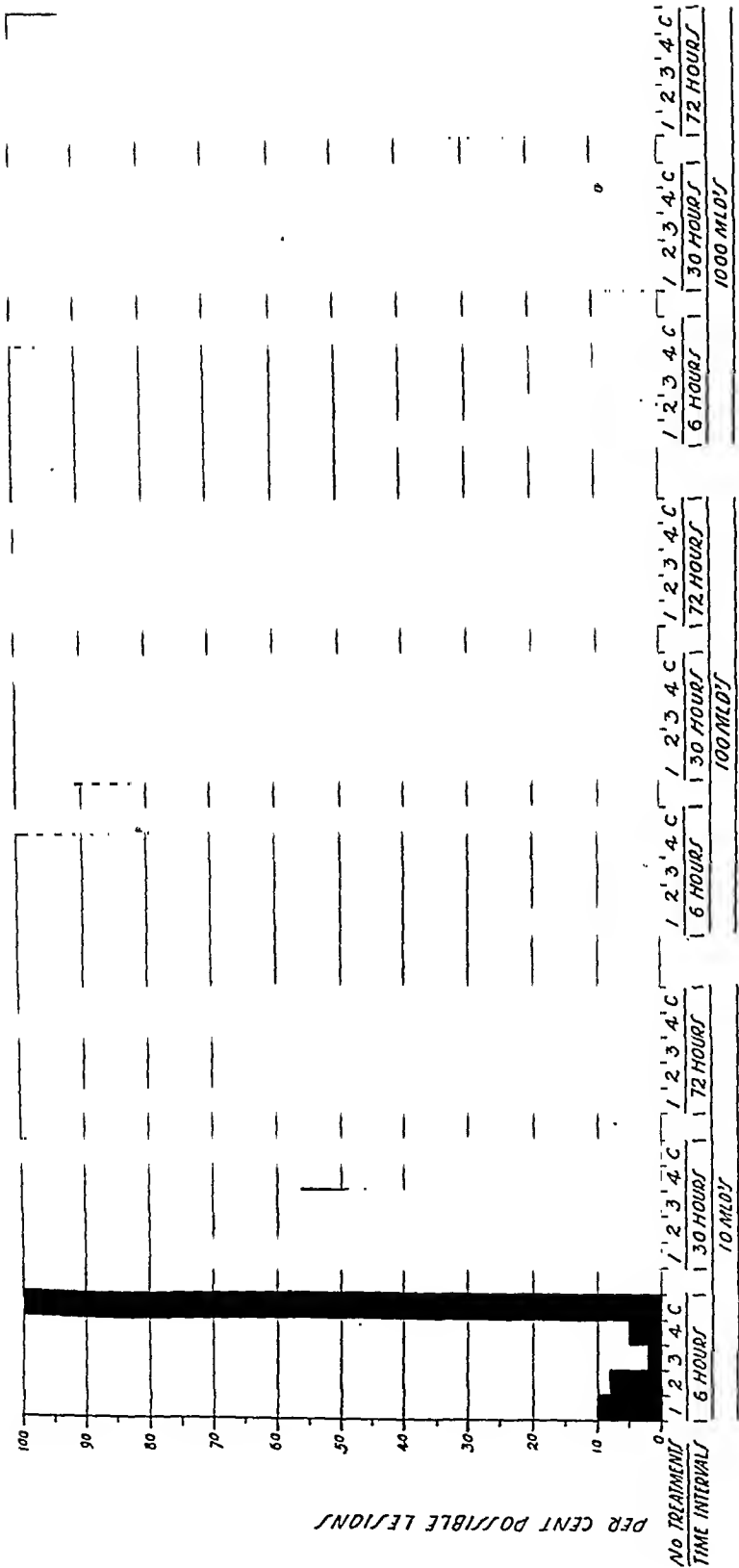


CHART 3.—Treatment of mice by intranasal inoculation of immune serum.

tion had progressed so far by the third or fourth serum instillation that the etherization and intranasal inoculation were not well tolerated. This is evidenced, for example, in the group receiving 10 MLD followed by 4 serum treatments starting at 72 hours. All the mice in this group died, due in great part to the repeated inoculations and administrations of anesthetic. The value of the successive serum treatments is clear, however, from the groups of mice in which treatment was begun earlier.

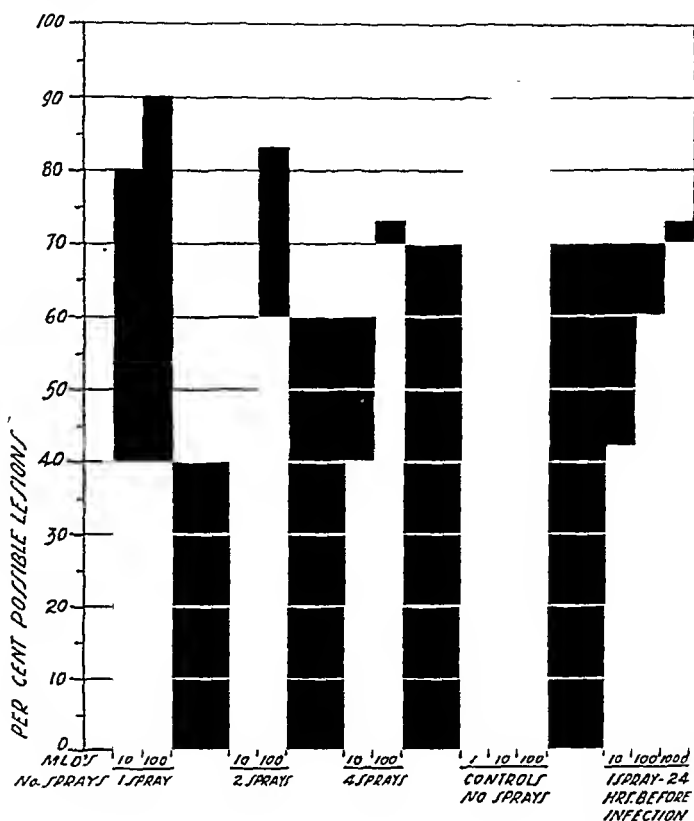


CHART 4.—Treatment of mice by inhalation of immune globulin.

Inhalation of immune globulin was also demonstrated to result in reduction of the lesion score in a group of mice which had been previously infected. Successive treatments were again shown to be more effective than a single spraying. Mice were inoculated with 10 or 100 MLD of PR-8 mouse lung virus 6 hours before spraying for 1 hour with a two and one-half-fold dilution of immune horse globulin. The mice were divided into groups receiving 1, 2 or 4 treatments, the multiple sprayings being spaced 24 hours apart. Ten days after the infecting dose all survivors were autopsied and lesions scored in the usual manner. Results are plotted in Chart 4 in terms of per cent of possible lesions. The decrease in lesion score with increase in the number of treatments, although not impressive, is consistent. Included for comparison are the results from a group of mice sprayed for 1 hour

with the same globulin solution and infected intranasally 24 hours later.

**Attempt at Immunization With a Neutral Mixture of Serum and Active Virus.** The consistent results obtained with serum in the prevention of experimental influenza by other workers as well as by ourselves led us to the possibility of using a serum-active virus combination in the hope that an active immunity would follow the short-lived passive immunity.

Preliminary experiments were performed in which mixtures of immune horse serum and allantoic fluid virus, non-infectious to mice upon intranasal inoculation, were instilled intranasally every 60 hours until 4 inoculations were made. Passive immunity was demonstrated 24 hours later and 2 weeks thereafter there seemed to be evidence of an active immunity having developed. In an attempt to confirm the results suggested in the preliminary work, the following experiment was performed.

Neutral mixtures of immune serum and PR-8 allantoic fluid virus, immune serum and Lee virus, and immune serum plus PR-8 and Lee viruses were incubated for 15 minutes at 37° C. The non-infectiousness of these mixtures was confirmed by mouse inoculation. A suspension of the serum plus normal allantoic fluid treated in the same manner was included as a control. Mice were inoculated with 0.05 cc. intranasally with one of the above mixtures every 48 hours until 4 inoculations were completed. Fourteen days later each group was tested for active immunity against both PR-8 and Lee mouse lung viruses by intranasal inoculation of from 10 to 10,000 MLD. Results were entirely negative and there was no evidence of an active immunity having developed.

The inability to produce an active immunity by combining serum with the virus is doubtless based upon the same mechanism noted in similar trials of combined vaccines against diphtheria and tetanus.<sup>19,22</sup> Although results are dependent to some extent on quantitative relationships and the time relationships between inoculations, the presence of a passive immunity, in general, tends to inhibit the normal development of an active immunity.

A later experiment designed to produce immunity in mice by intranasal inoculation of a formolized vaccine gave further confirmation of these results. Mice were divided into 2 test groups, the first group receiving 0.05 cc. of formolized PR-8 allantoic fluid virus intranasally every other day until 5 inoculations were completed, the second group receiving the same course of formolized virus inoculations and also being inoculated with 0.05 cc. of immune horse serum intranasally on the alternate days. This second group of mice thus received 5 formolized virus inoculations and 4 serum inoculations. Ten days after the last inoculation both treated groups, as well as control mice of the same age, were tested for active immunity by intranasal inoculation of PR-8 mouse lung virus. The results are presented in Chart 5 and demonstrate the definite establishment of immunity in mice inoculated intranasally with inactive virus, but a lack of immunity in the mice



which alternately received serum and vaccine. Although it has been demonstrated by Eaton and Beck<sup>8</sup> that with active mouse-adapted strains of virus inoculated intranasally it is necessary to produce rather severe lung lesions in order to induce an immune state, an inactive virus

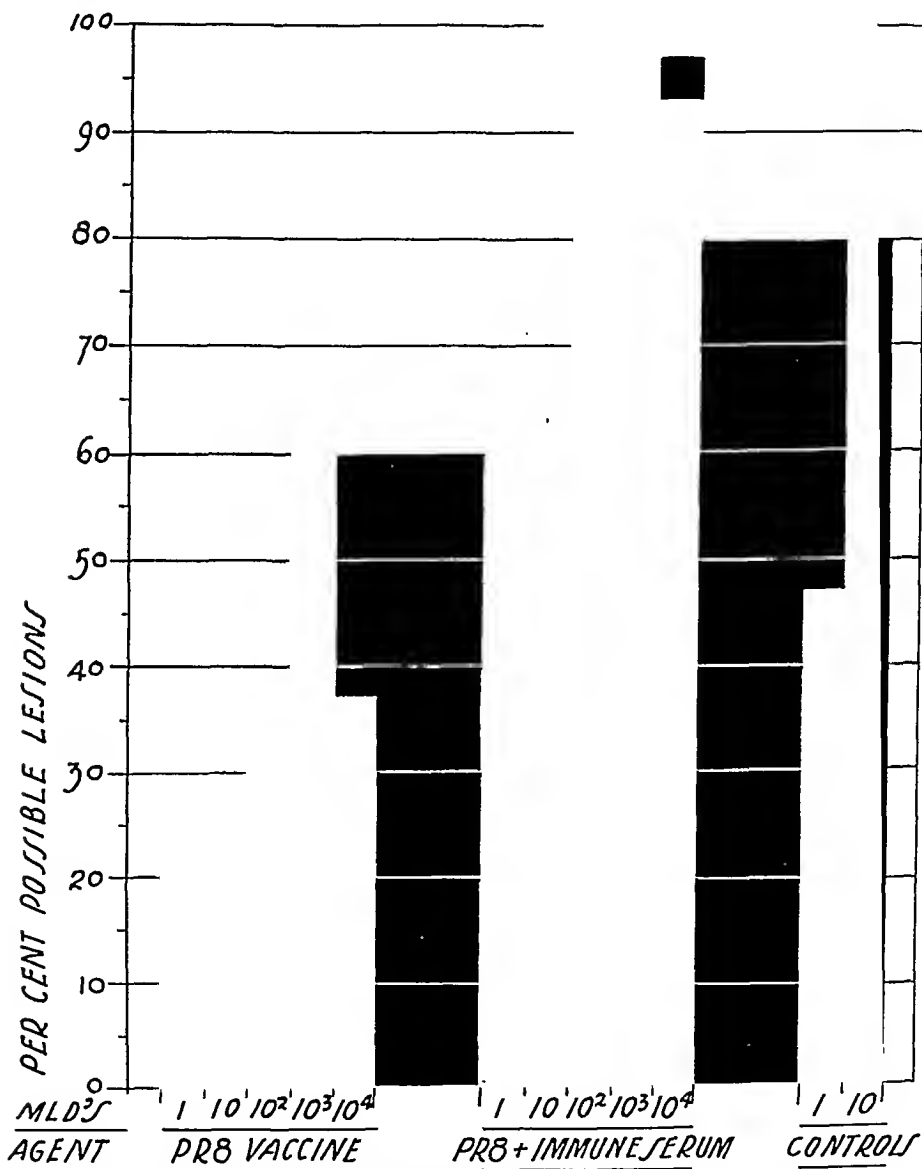


CHART 5.—Immunization of mice by intranasal inoculation of formalized virus alone or in combination with immune serum.

is capable of immunizing mice intranasally with no injurious effects. The inhalation of inactive virus by mice would very likely result in even better immunization because of the more uniform distribution of the antigenic material. This aspect of the problem is now under investigation.

The reports of Walsh and Cannon<sup>5,34,35</sup> are of interest in this regard, for they indicate that the ratio of tissue antibodies to serum antibodies is consistently higher following intranasal vaccination than that resulting from a general immunization. Their antigens were bacterial in nature, but it might be anticipated that the same principle would apply in the case of influenza virus. Their belief is that local formation of antibodies is responsible for the higher concentrations found in organs of the regionally immunized animals and that regional vaccination would seem therefore the preferable method for elevating the level of resistance of tissues at a portal of entry to the body. Therefore, the direct administration of an influenza vaccine to the respiratory tract should be carefully considered as a possible method of choice. The question of the possible use of attenuated virus to produce a sub-clinical infection as a method of immunization has been discussed recently by Francis<sup>10</sup> and Burnet and Clark.<sup>3</sup>

**Summary.** 1. High titer horse immune serum or its globulin fraction, administered either by intranasal inoculation or by inhalation, protected mice against subsequent intranasal infection with influenza virus. The degree of protection conferred increased with the time of exposure to the globulin spray.

2. Atomization of solutions through nebulizers of the type employed results in a distillation effect, which must be taken into consideration in quantitative estimates of inhaled materials.

3. Whole immune plasma was superior to any individual globulin fraction in the degree and duration of its protective power for mice.

4. Treatment of mice with horse immune serum intranasally, or globulin by inhalation, is effective in reducing the lung lesions. The necessity for early treatment is confirmed. The value of repeated treatments in lessening the severity of the experimental disease is definitely established, the lung lesions decreasing as the number of treatments is increased.

5. Neutral mixtures of immune serum and active virus were ineffective in producing an active immunity in mice.

6. Mice subjected to repeated intranasal inoculations of a formolized virus showed a considerable degree of immunity when tested 10 days following the last inoculation. Immunity failed to develop in mice receiving concurrent intranasal serum treatments along with the formolized virus.

7. The prospective application of passive immunization of humans for the prophylaxis and treatment of humans is discussed.

**ADDENDUM.**—Considerable newspaper and magazine publicity containing inaccurate and unwarranted claims relative to our findings has preceded publication of this article. The authors were in no way responsible for this and take this means of expressing their disapproval of the resultant publicity.

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## YEAST-LIKE FUNGI IN THE INTESTINAL TRACT OF CHRONICALLY INSTITUTIONALIZED PATIENTS

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THE paramount problem confronting the bacteriologist in institutions with notably large bed capacity, such as state hospitals or penitentiaries, is the control of enteric infections.

It is a matter of regret that both for the therapy and prevention of such enteric disturbances, the finding of bacteria or parasites commonly considered as pathogenic does not provide at all times a secure guide. The negative findings of the usual clinical and microbiologic examinations in many cases of diarrhea prompted us to check not only the "pathogenicity" of intestinal microorganisms, but also to extend

our efforts to the search for possible new agents. Investigating the question of mycotic infections, it was found that little work had been conducted in the field of enteric mycology within the past few years. We therefore considered it our first task to investigate the "normal" flora of the intestinal tract in a large number of institutional patients.

Our main interest was concentrated on the *Monilia*-*Cryptococcus*-*Geotrichum* group. The *Penicillium* and *Aspergillus* species were neglected because contamination of the specimens could not be fully precluded. The simple and efficient method of Schnoor<sup>3</sup> as well as the nomenclature adopted by him were used. Thus we followed the classification of *Moniliae* according to Martin *et al.*<sup>2</sup> Several attempts were made to differentiate *Cryptococcus* strains according to Benham and Hopkins<sup>1</sup> into 4 groups. The morphology of the colony, however, did not seem to be a sufficient guide. We found that due to the lack of serologic means a classification of these organisms was not possible. *Torula*, *Saccharomyces* and similar organisms were only occasionally encountered, and are not reported in this paper. Only 11 such strains scattered throughout the examined groups were cultivated.

Three groups of hospital patients were selected for the investigation, none of whom were afflicted with a skin disease. The stool specimens were collected from patients of the Manteno and Chicago State Hospitals.

1. The first group of cases included the stool specimens from 300 newly admitted patients and 600 cases from chronic wards, all of them without intestinal disturbances. This material was chosen in order to determine whether the intestinal flora changed during the hospitalization.

2. The second group consisted of specimens from 100 chronic typhoid carriers.

3. The third group was composed of diarrhea cases. (a) Stool specimens from 47 patients with *Shigella sonnei* and *Shigella paradysenteriae* infections in the early acute stage. (b) Stool specimens from 51 proctoscopically confirmed\* cases of bacillary dysentery 1 week after the termination of the sulfathiazole treatment. (c) Finally, the stool specimens from 103 cases of "food upset" occurring in 3 groups. This latter series consisted of patients who had shown a short period of diarrhea (from 4 to 8 hours) with only a few bowel movements, revealing light-colored stool without blood, mucous or other pathologic constituents. Bacteriologic, parasitologic and proctoscopic examination failed to disclose the cause of the upsets.

The results of the examination may be found in Table 1.

In comparison with the findings of Schwartz and Jankelson<sup>4</sup> and Schnoor<sup>3</sup> a higher percentage of *Monilia* and a lower percentage of *Geotrichum* were found in patients newly admitted to the hospitals. There were only a few patients who harbored 2 species of the group. In cases hospitalized for a longer period of time the incidence of *Monilia*, *Cryptococcus* and *Geotrichum* increased in the following man-

\* The proctoscopic examinations were performed by Dr. L. H. Block, Consultant Proctologist of the Illinois Department of Public Welfare, Hospital Service.

ner: The number of cases excreting these organisms did not increase essentially, but many cases already harboring 1 member of the group became infected with a second or eventually also with a third species. In the absence of proper serologic tests, it is difficult to decide what immunologic process determines one person to be a ready host and a second person a less favorable host to these organisms.

TABLE 1.—ANALYSIS OF FUNGI FOUND IN DIFFERENT TYPES OF PATIENTS

Fungi found	Types of cases examined											
	New admissions		Ward cases		Typhoid carriers		Food upset		Bacillary dysentery		Treated dysentery	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of cases:	300		600		100		103		47		51	
<i>Monilia albicans</i>	58	19.3	226	37.7	34	34	42	40.8	21	44.6	6	12
<i>parapsilesis</i>	13	4.3	26	4.3	3	3	1	0.9	2	4.2	1	2
<i>krusei</i>	7	2.3	16	2.7	4	4	1	1.9	1	2.1	0	0
others	1	0.3	3	0.5	2	2	2	1.9	2	4.2	0	0
<i>Monilia</i>	79	26.3	271	45.1	43	43	47	45.6	26	55.3	7	14
<i>Cryptococcus</i>	72	24.0	235	39.2	35	35	36	34.9	15	34.9	6	12
<i>Geotrichum</i>	69	23.0	177	29.1	26	26	58	56.3	25	53.1	3	6
Distribution												
Negative	80	26.6	146	24.6	26	26	15	14.6	8	17.0	37	74
1 species present	206	68.6	272	45.3	48	48	38	36.8	17	36.1	10	20
2 species present	14	4.7	155	25.8	22	22	47	45.6	17	36.1	3	6
3 species present	0	0	27	4.5	4	4	3	3.9	5	10.6	0	0

Typhoid carriers did not present a noticeable difference from the "chronic ward" patients.

In the "food upset" cases the incidence of *Geotrichum* increased doubly. The increased number of patients infected with *Geotrichum* reduced the number of cases without infection. Nearly 50% of the patients harbored 2 organisms. There was no change in the incidence of *Monilia* and *Cryptococcus*.

Although the low number of cases with bacillary dysentery does not permit definite conclusions, it must be conceded that the statistics differ from "food upsets" by the increase of *Monilia* findings and by the rise of the number of patients harboring 3 organisms.

In cases of dysentery treated with sulfathiazole, the absolute and relative incidence of *Monilia*, *Cryptococcus* and *Geotrichum* decreased substantially. Statistically, *Geotrichum* disappeared in seven-eighths of the cases, *Monilia* in approximately two-thirds, and *Cryptococcus* in about five-eighths—an observation which may possibly prove of some value in the treatment of mycotic diseases.

**Summary.** *Monilia*, *Cryptococcus* and *Geotrichum* were studied in stool specimens of 300 new admissions, 600 chronic ward patients, 100 typhoid carriers, 103 cases of "food upset," 47 acute bacillary dysenteries before treatment, and in 51 cases of bacillary dysentery after 10 days of sulfathiazole treatment. During the hospitalization the number of patients harboring the aforementioned organisms did not

increase but many cases previously infested only with 1 organism developed into carriers of 2 organisms. Typhoid carriers did not show a differing picture. In "food upset" the number of cases with *Geotrichum* increased remarkably. In bacillary dysentery the number of patients with *Monilia* also rose. Sulfa treatment reduced the number of mycotic carriers, and especially those with *Geotrichum*, by a pronounced margin.

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## THE NATURALLY OCCURRING BLOOD SULFOCYANATES AND THEIR RELATION TO BLOOD PRESSURE

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IN 1925 Nichols<sup>4</sup> discussed the pharmacologic properties of the sulfocyanates. He regarded the salivary glands as their chief site of formation, from which they were secreted into the saliva, swallowed and absorbed into the "system" appearing in tears and gastric juice. The salivary content of sulfocyanates averaged 0.01%. He assumed that if 1 liter of saliva was formed in 24 hours the amount of sulfocyanates are found in blood and body fluids in fairly equal concentration varying from 0.03 to 0.06 mg. per 100 cc. and excreted in the urine and feces in the ratio of 1:2. Cerebrospinal fluid concentrations are either the same or lower than in the blood. Caviness and his associates<sup>2,3</sup> have recently reinvestigated the naturally occurring sulfocyanates in blood using a technique modified from Ravin<sup>5</sup> which revealed concentrations definitely higher (0.31 to 2.55 mg. KCNS per 100 cc. blood) than had been previously reported. Repetition of determinations in the same subject exhibited a marked constancy of sulfocyanate concentrations. In 241 individuals studied, the blood sulfocyanate concentration varied inversely with the height of the blood pressure. They therefore ventured in hypothesis that naturally occurring blood sulfocyanates function as depressor substances which serve to counterbalance the action of various naturally occurring pressor substances, thus representing one of the many factors concerned in the maintenance of normal blood pressure. The importance of these observations, which would necessarily modify our concept of the pathogenesis of hypertension, stimulated us to undertake the work to be reported.

**Methods.** We studied 95 hospital in-patients and, as subjects of the tobacco experiment several volunteer residents, internes, and nurses.

Blood pressure recordings (a mercury column or anaëroid sphygmomanometer was used in measuring the height of the blood pressure) represent the average of 3 or more readings on different days with the subject at complete bed rest for 10 minutes or longer. Subjects with very obese or very thin arms, those whose blood pressures varied appreciably on successive readings, and patients in cardiac decompensation, in shock, or the victims of myocardial infarction, or patients suffering with febrile diseases were excluded from the study because of obvious sources of error. The diastolic levels recorded are those of Phase IV.<sup>1</sup>

Caviness' criteria for grouping subjects according to blood pressure levels were followed with a single modification:

- Below 106 systolic: hypotension;
- 106 to 130 systolic: normotension;
- 131 to 140 systolic: borderline;
- 141 to 200 systolic: hypertension;
- 200 and above systolic: severe hypertension.

Caviness<sup>2,3</sup> stressed the necessity of control of physical activity, rest and diet in order to obtain accurate and comparable results in different patients. He collected blood samples before breakfast and before any tobacco was used that day. We adhered to these criteria, but, in addition, found that it was not sufficient to interdict the use of tobacco to the period immediately preceding the drawing of blood because habitual tobacco smokers show definitely higher blood sulfocyanate levels than non-smokers. Therefore our blood samples were all collected at least 48 hours or more after the use of tobacco.

**Technique.** To approximately 10 cc. of blood serum an equal amount of 10% trichloroacetic acid was added, thoroughly admixed, and allowed to stand for 20 minutes. To 5 cc. of the protein-free filtrate obtained by filtering this mixture, 1 cc. of 5% ferric nitrate solution was added and the sulfocyanate concentration in milligrams per 100 cc. of blood was read directly in an Evelyn photoelectric colorimeter using a filter wave length 440 m.<sup>2,3</sup> We also checked the accuracy of our results by recovery tests upon several occasions during the period of this work.

**Results.** In 34 subjects with normal blood pressure (average pressure 116/71) the average blood sulfocyanate concentration was 1.31 mg. KCNS per 100 cc. of blood with a range of 0.84 to 2.4 mg. In 20 hypotensive subjects (average B.P. 93/58) the average blood sulfocyanate concentration was 1.18 mg. (range 0.90 to 1.54). In the hypertensive group (average B.P. 178/98) 28 subjects showed an average concentration of 1.19 mg. (range 0.74 to 2.48). Ten patients were included in the severely hypertensive group (average B.P. 227/120); they exhibited an average sulfocyanate concentration of 1.12 mg. (range 0.76 to 1.50); only 3 patients were investigated in the borderline blood pressure group (average B.P. 137/88) and presented concentrations of 1.21, 1.65 and 0.96 mg. (average of 1.24). A graphic comparison of these findings with those of Caviness *et al.* is demonstrated in Figure 1.

The effect of tobacco smoking on the blood sulfocyanate concentration was determined. Eight normotensive non-smokers served as a control group with an average sulfocyanate level of 1.03 mg. (range 0.84 to 1.21). The blood sulfocyanate level in 8 habitual tobacco smokers with normal blood pressures was 1.91 (range 1.24 to 2.48 mg.). One of these subjects smoked an average of 4 cigarettes per day and her sulfocyanate concentration was 1.24, the lowest recorded for the

tobacco habitués. Another subject smoked 8 cigarettes daily and showed a concentration of 1.45 mg. The remaining 6 subjects smoked 20 or more cigarettes per day. Blood samples on the 8 tobacco smokers were obtained after a 24 hour period of abstinence from smoking.

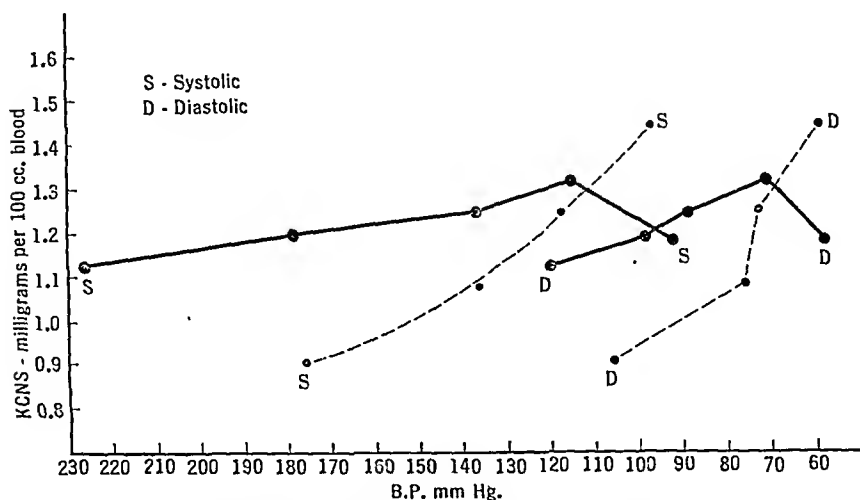


FIG. 1.—Blood pressure levels and blood sulfocyanate concentrations. Dotted lines represent the results of Caviness *et al.*; solid lines represent our results.

Excessive smoking for a 24 hour period produced no significant change in the sulfocyanate concentrations of these 3 subjects.

TABLE 1.—LACK OF EFFECT OF HEAVY SMOKING

	After 24 hr. period of tobacco abstinence	After 24 hr. period of heavy smoking (30-40 cigs.)
Subject A . . . . .	1.75	1.80
Subject B . . . . .	2.55	2.25
Subject C . . . . .	1.90	1.91

The effect of a high-protein intake after a varying period on a low-protein diet was investigated in 2 cardiac patients. After an initial blood sulfocyanate concentration was obtained, these 2 patients were given a 100 gm. protein diet and 200 cc. of 15% amino acids intravenously daily. The results are tabulated below:

TABLE 2.—EFFECT OF CHANGES IN AMOUNT OF PROTEIN IN DIET

	Initial KCNS	48 hrs. on high-protein intake	7 days
Subject D . . . . .	1.21	1.07	0.98
Subject E . . . . .	1.44	1.29	1.24

Repetition of the sulfocyanate determination was done in 16 patients on different days. The greatest difference in any two determinations on the same patient was 0.37 mg.; the smallest difference was 0.01 (average difference for the group 0.16). In 5 subjects, 3 or more repetitions were performed which revealed a similar constancy of sulfocyanate concentrations in successive determinations.

**Discussion.** The blood concentration of the sulfocyanates obtained by us in normotensive subjects are almost identical with the values



reported by Caviness and his associates. They found an average concentration of 1.21 mg. KCNS per 100 cc. of blood (range 0.31 to 2.55); our average value was 1.31 mg. (range 0.84 to 2.40 mg.). We also noted a marked constancy of values in successive determinations in the same subject. We found no constant relationship, however, between the height of the blood pressure and the concentration of blood sulfocyanates, whereas Caviness demonstrated a definite inverse ratio existing between the two. In Figure 1 the discrepancy of results in the two series of experiments is graphically illustrated. The almost vertical lines that we obtained demonstrate the complete lack of correlation of blood sulfocyanate concentration and the height of the blood pressure in our hands.

We attempted to control our experiments identically with the conditions that obtained in their work as regards environmental and dietary factors and technique of chemical manipulations. Though most of our subjects were patients hospitalized for various reasons and theirs were apparently normal institutional inmates and prisoners, we cannot believe this to be the determining factor to explain our failure to confirm their reported results. We have demonstrated the uniformly elevated sulfocyanate blood levels in habitual tobacco smokers as compared to a control group of non-tobacco users. Caviness and his co-workers stated that their only precaution as regards tobacco smoking was to draw blood samples in the morning "before tobacco was used." Since we found that blood sulfocyanate levels remain elevated in tobacco smokers after a 24 hour period of abstinence this precaution is not sufficient to eliminate the effect of tobacco in habitual smokers. This may, in part, explain the elevated sulfocyanate blood concentrations in some of their subjects.

In the light of our findings we cannot regard the blood sulfocyanates as naturally occurring depressor substances nor can we regard sulfocyanate therapy in hypertension as a form of substitution therapy for a substance that, because of a deficiency in the blood, has allowed various pressor substances to act uninhibited resulting in hypertension.

**Summary.** 1. Sulfocyanates occurred normally in human blood in concentrations averaging 1.31 mg. per 100 cc.

2. Habitual tobacco smokers showed blood sulfocyanate concentrations well above the normal average.

3. No constant relationship of blood pressure to blood sulfocyanate concentration was found.

4. The naturally occurring blood sulfocyanates probably do not represent an important factor in the maintenance of normal blood pressure.

We wish to thank Dr. David R. Meranze, Director of the Laboratories, for the use of the Laboratory Facilities, and Joseph Schaffer, B.A. (Chem.) and Herbert Marks, B.A. (Chem.) for performing the sulfocyanate determinations.

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## TREATMENT OF 134 CASES OF MENINGOCOCCIC INFECTION WITH MASSIVE DOSES OF SULFADIAZINE

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THE treatment of meningococcic infections has undergone considerable and important changes since the introduction of the sulfonamide drugs. Palliative treatment was the keynote of therapy after the isolation of the meningococcus by Weichselbaum in 1887 until Flexner introduced serum therapy in 1908. The use of antimeningococcus serum intravenously, intramuscularly, and often intrathecally, in conjunction with supportive and symptomatic measures, held unchallenged sway until 1936, when the sulfa drugs—sulfanilamide followed by sulfapyridine, and sulfadiazine—were demonstrated to be efficacious additions to the therapeutic armamentarium of meningococcic infections. Nevertheless, there is no uniformity of opinion as to the relative merits of serum and the sulfonamides; some investigators recommending the use of sulfonamides alone, others, a combination of chemo- and serotherapy.

During the pre-serum stage in the treatment of cerebrospinal fever, the mortality rate varied from 20 to 90%. Following the use of serum, mortality figures declined, ranging between 20 to 40% in most series reported.<sup>9</sup> During the first World War 5839 cases of meningococcic meningitis were reported by the Office of The Surgeon General with 2279 fatalities, a mortality incidence of 39%.<sup>11</sup> The discovery of sulfanilamide in 1936, sulfapyridine in 1938 and sulfadiazine in 1940 ushered in the third or chemotherapeutic phase in the treatment of meningococcic diseases. Dingle and Finland<sup>3</sup> presented an extensive review of the literature of meningococcic meningitis with comparative mortality rates for different combinations of therapy. In over 1000 cases treated with sulfanilamide alone, the mortality rate was 14%; in 300 patients treated with combinations of sulfanilamide and antiserum, 25%; in approximately 700 patients treated with sulfapyridine alone, 8%; and for 200 patients treated with sulfapyridine and serum, 12%. The number of patients treated with sulfadiazine was insufficient to permit appraisal and comparison with other sulfonamide drugs. The reason for the higher mortality rates when sero- and chemotherapy were used in combination seemed difficult to explain and evaluate. One plausible explanation is that this group of patients was more

seriously ill or had failed to respond to the sulfonamide drugs alone. On the other hand, mortality figures in meningococcie infections have been consistently lower when treated by a combination of antiscrum and one of the sulfa group than by the use of antiscrum alone. Campbell<sup>2</sup> reported a series of 10 cases treated with scrum and sulfanilamide with no deaths. This series is too small in itself to be of any statistical significance. Banks'<sup>1</sup> series of 65 cases were treated with sulfanilamide, terminating in 8 deaths, (12.3% mortality). In a recent editorial on the therapy of cerebrospinal fever in England,<sup>4</sup> the following statistics in all age groups were reported: treatment with scrum alone gave a fatality of 36.6%; with sulfonamide compounds plus scrum, 13.8%; with sulfonamide compounds alone, 9.2%.

The present report is an analysis of 134 consecutive cases of meningococcie infections admitted to the Station Hospital, Fort McClellan, Alabama, since March 13, 1942. This series occurred in two separate epidemic waves. The first epidemic of 15 cases occurred between March 13 and May 6, 1942. The second epidemic which began on December 7, 1942, consisted of 119 cases, and includes all cases up to April 16, 1943.

The criteria for establishing a diagnosis of meningococcie meningitis were: (1) Clinical signs of involvement of the central nervous system, such as nuchal rigidity, abnormal neurologic reflexes, *e. g.*, Kernig and Brudzinski signs and state of orientation and consciousness. (2) Confirmatory laboratory evidence of meningitic spinal fluid changes, with bacteriologic smears and cultures.

Despite the fact that several patients in the first epidemic were critically ill, and admitted to the hospital in coma, no fatalities occurred. The second epidemic presented a definitely more virulent type of infection. These cases were characterized by a stormier clinical course, with rapid progression until adequately treated.

All cases were males in previous good health, varying in age from 18 to 44. Ten patients in our series were colored, and 124 white, of which 44% were between the ages of 21 and 30. (Fig. 1.)

FIG. 1.—CLINICAL AND LABORATORY FEATURES

I. Race:		V. Blood cultures:	
White . . . . .	124	Positive . . . . .	42
Colored . . . . .	10	Negative . . . . .	89
II. Age distribution:		Not taken . . . . .	*3
18-20 yrs. . . . .	58	VI. Lumbar puncture:	
21-30 yrs. . . . .	59	Clear fluid . . . . .	55
31-40 yrs. . . . .	15	Cloudy fluid . . . . .	79
41-50 yrs. . . . .	2	VII. Sulfa drug used:	
III. Skin rash:		A. Sulfadiazine . . . . .	109
Petechiae . . . . .	96	B. Sulfanilamide . . . . .	3
Purpura . . . . .	9	C. Sulfadiazine and Sul-	
No eruption . . . . .	29	fanilamide . . . . .	17
IV. Orientation:		D. Untreated . . . . .	*5
Conscious . . . . .	82	VIII. Results:	
Stuporous . . . . .	31	Recovered . . . . .	125
Comatose . . . . .	21	Died . . . . .	9
Total per cent mortality . . . . .		6.7%	
Mortality of treated cases . . . . .		3.1%	

\* Fulminating cases which expired within 12 hours after admission. (See text.)

These cases were fairly well distributed among the various units except for a large body of seasoned negro troops in which only one case appeared. This apparent paradox can best be explained on the grounds that the former were green, raw recruits while the latter were trained, seasoned, conditioned troops in which the incidence of upper respiratory infections was at a minimum. The percentage of negroes among the unseasoned troops was relatively low.

The sulfa drug employed in this series was sulfadiazine, but sulfanilamide was used in some cases (v.i.). Neither sulfapyridine nor sulfathiazole was employed in this series because of their more frequent toxic manifestations and the poor excretion of sulfathiazole into the spinal fluid.

The dosage of sulfadiazine in this series was much larger than that ordinarily recommended. Our basic plan of dosage was as follows: initial dose, 8 gm., second dose (2 hours later), 5 gm.; third and fourth doses (at 4 hour intervals) 4 gm.; subsequent doses, 3 gm. every 4 hours. This régime was practically constant in the first 24 hours. The blood sulfadiazine level was determined 18 to 24 hours after therapy had been initiated and daily thereafter, the subsequent dosages of sulfadiazine being regulated accordingly. We regarded the optimal blood sulfadiazine concentration to be between 15 to 20 mg. per 100 cc. If the blood sulfadiazine failed to attain satisfactory levels after the 1st day of chemotherapy, the dosage was "stepped up" to 4 gm. for one or two doses and then decreased to what we considered a maintenance dose—2 gm. every 4 hours and, in some instances, 2 gm. alternating with 1 gm. every 4 hours. Daily blood sulfadiazine levels, clinical appearance of the patient and absence of toxic manifestations were our dosage criteria.

In severe or fulminating meningococcic infections the intravenous administration of a 5% solution of sodium sulfadiazine was the initial procedure, since in these cases, it was imperative to obtain the optimal blood sulfadiazine level rapidly. The same route was used in instances of persistent vomiting. The medication was prepared by dissolving 5.5 gm. of sodium sulfadiazine in 111 cc. of sterile distilled water. Glucose in saline was usually given intravenously just prior to, or following, this medication. Immediately following the administration of the sodium salt intravenously, the basic plan of dosage described above was instituted. In stuporous or unconscious patients the oral drug was administered by Levin tube.

Sulfanilamide was administered in smaller doses than sulfadiazine as the optimum blood concentration of the former was regarded to be 12 to 15 mg. The initial dose of sulfanilamide was 4 gm. repeated in 2 hours and followed by 1 gm. every 4 hours for the first 24 hours of therapy. Dosage was then augmented or diminished, depending upon the blood sulfanilamide level. One gram of sodium bicarbonate was given routinely with each dose of sulfanilamide because of the frequency of acidosis with this drug. The substitution of sulfanilamide was reserved for those cases with renal complications due to sulfadiazine in which the sulfadiazine therapy up to that time had been inadequate.

It was difficult to determine when sufficient sulfadiazine therapy had been administered. There was no set rule or standard on which discontinuance of the drug could be based. Maintenance of therapy was largely guided by several factors—primarily the clinical course of the patient as well as the blood count, and by the presence or absence of toxic drug reactions. Temperature in our experience was notoriously inaccurate and unreliable as a guide.

In our series chemotherapy was discontinued only in the presence of the following toxic manifestations: hemolytic anemia, oliguria, anuria, and hematuria. The remaining complications—drug fever, cyanosis and renal colic—were not believed to be adequate contraindications for further use of the drug. The occurrence of renal colic, however, made us cautious in the further use of sulfadiazine but in itself was not felt to be sufficient reason for discontinuing therapy. The urinary output and fluid intake were closely watched in all cases, to insure satisfactory renal excretion. Daily specimens of urine were examined to detect any signs of kidney damage.

**Discussion.** The purpose of this presentation is to demonstrate the effect of massive doses of sulfadiazine on the course and outcome of a series of 134 consecutive cases of meningococcal infection. The clinical and laboratory features of the total series are summarized in Figure 1. There were 97 cases of meningitis and 18 of meningococemia confirmed by laboratory and bacteriologic evidence. In the remaining 19 cases of meningococemia no organisms could be found on smear or culture, but sufficient clinical and laboratory data existed to substantiate the diagnosis.

We have utilized doses of sulfadiazine much greater than ordinarily employed. Initial oral doses of 8 gm. were employed as contrasted with ordinarily recommended doses of 4 to 5 gm. Subsequent doses for the first 24 hours ranged between 3 to 4 gm. every 4 hours. Our average total dose of sulfadiazine for the first 24 hours was 25 gm. During the second day of treatment, the average total quantity of drug given varied between 12 and 15 gm. but this was not constant and was increased if an optimum blood concentration of sulfadiazine was not reached. The total amounts of sulfadiazine given to individual patients fluctuated widely. In our series, the largest total dose employed was 245 gm. and the lowest total dose 50 gm.: average approximately 68 gm. Factors involving absorption of the drug from the intestinal tract and rapidity of renal excretion or elimination must be considered in explaining these wide variations. Moreover, the severity of the infection, duration or stage of the disease, time of institution of therapy, varied clinical response of the individual to the drug, age of the patient, and time of appearance of toxic drug reactions materially affected the total dosage administered.

Of the 134 consecutive cases of meningococcal infection reported in this paper, a total mortality of 9 cases (6.7%) for the entire group resulted. All fatal cases were autopsied. However, 5 of these cases were inadequately treated and died within 12 hours of admission. An analysis of these 5 cases showed that 2 were fulminating meningococ-

cemias with the Waterhouse-Friederichsen syndrome; 3 cases were meningitis, in 2 of which the *Micrococcus catarrhalis* was isolated both culturally and by sugar fermentation tests. These 5 cases, therefore, cannot be included in the group of treated cases. Adequate therapy was defined as treatment with sulfadiazine or sulfanilamide over a minimal period of 24 hours. Four fatalities occurred in the remaining 129 patients who received adequate therapy, a treated mortality rate of 3.1%. Three of these cases exhibited meningococcemia with the Waterhouse-Friederichsen syndrome and the 4th case had a meningococcic meningitis.



FIG. 2.—Smear of peripheral blood, demonstrating intracellular diplococci, and toxic degeneration of leukocytes. (Courtesy, Signal Corps, United States Army.)  $\times 440$ .

The Waterhouse-Friederichsen syndrome accounted for 5 of the 9 deaths in our series. This syndrome is characterized by petechial or purpuric skin lesions and an overwhelming septicemia associated with massive adrenal hemorrhages. This condition is rare in adults: 70% of the recorded cases having been noted in children under 2 years of age. These 5 cases bring to 101 the total of reported cases in the literature up to the present time.<sup>7</sup> The survival periods of our cases of the Waterhouse-Friederichsen syndrome varied between 1 and 88 hours. The shortest-lived case (1 hour) showed Gram-negative diplo-

eocci in a routine blood smear (Fig. 2). On microscopic section, all revealed moderate to severe adrenal hemorrhages (Fig. 3).

The toxic effects of sulfanilamide and sulfadiazine encountered in this group are presented in Figures 4 and 5. The complications due to sulfanilamide therapy were in agreement with those reported by other observers. Eight out of 20 cases, (40%) treated with sulfanilamide had one or more toxic manifestations. Sulfanilamide was discontinued in the presence of hemolytic anemia and drug fever. We did not feel that the other drug reactions, acidosis and cyanosis, warranted discontinuance of chemotherapy. The 2 cases of hemolytic anemias encountered

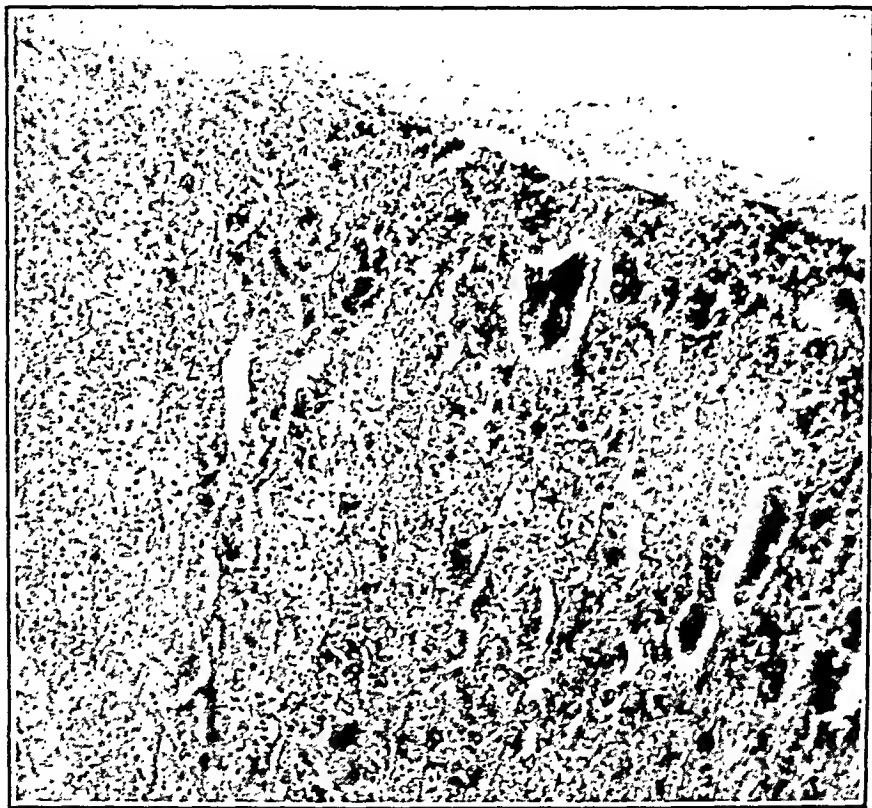


FIG. 3.—Section of adrenal gland showing massive interstitial hemorrhages in cortex and medulla, with few remaining islands of normal tissue. (Courtesy, Signal Corps, United States Army.)  $\times 440$ .

ered were of a mild type and did not require blood transfusions. The temperature promptly dropped to normal in the drug fever group upon discontinuing the drug.

Drug toxicity with sulfadiazine was encountered more frequently than has been reported by other observers. The most serious toxic effects involved primarily the kidneys. In this series of cases complications of all types due to sulfadiazine reached a total of 28%. Of all complications 86% was renal in character. This is in marked contrast to the results in a series of over 300 cases of pneumonia treated with sulfadiazine cited by Ratish, Shackman and Bullowa<sup>10</sup> in which gross

hematuria occurred in only 3 cases (an incidence of 1%). No mention is made, however, of the frequency of microscopic hematuria. Finland, Peterson and Goodwin<sup>5</sup> found gross and microscopic hematuria in 5.2% of a series of 460 patients treated with sulfadiazine. Only a small group of these cases were of meningococcic infection, and the group, as a whole, required comparatively small doses of sulfadiazine.

We did not regard renal colic an indication for stopping sulfadiazine chemotherapy, as recommended by Keitzer and Campbell.<sup>7</sup> We continued the use of the drug cautiously, exercising great care in its administration. Six cases of renal colic were encountered, 2 of which developed oliguria, and 1, anuria. The remaining 3 cases showed no further evidence of renal irritation, despite the presence of renal colic.

Anuria is the most serious complication of sulfadiazine medication and fatalities have been reported.<sup>6</sup> One of our cases developed an anuria lasting 19 hours. This patient was treated conservatively. Fluids were forced by all routes and renal function was reestablished.

Drug fever is an unusual complication of sulfadiazine medication, but the occurrence of sulfadiazine arthritis is even rarer. Due to our limited facilities we have not been able to make an exhaustive review of the literature on this latter subject. Drug fever with an incidence of 0.2% was reported by Finland *et al.*<sup>5</sup> in their series of 460 cases, but no cases of arthritis or arthralgia occurred in the same group. Arthritic manifestations attributable directly to sulfadiazine therapy occurred in 2 of our cases, in one of which drug fever was also present. The following case is cited because of its unusual interest and rarity.

**Case Study.** CASE 1 (Fig. 6). F. T. M., a 35 year old white male was admitted to the hospital on January 11, 1943, in an unconscious state, because of chills, vomiting, headache, pains in the joints and soreness of the muscles of 24 hours duration. On examination, patient was comatose and the skin covered with petechiae. He presented the classical signs of a meningitis with nuchal rigidity and positive Kernig and Brudzinski. The pharynx was not injected. No spleen was palpable. Temperature on admission was 101.6° F., pulse 132, respiration 28.

A lumbar puncture showed a cloudy fluid under increased pressure containing 14,800 white blood cells with 95% polymorphonuclears. Globulin was positive. Smear revealed many extra- and intracellular Gram-negative diplococci. Blood and spinal fluid cultures were negative. The white blood count was 36,550 cells with 89% polymorphonuclears. A Levin tube was passed and an initial dose of 8 gm. of sulfadiazine given. Within the first 24 hours, the patient received 25 gm. of sulfadiazine orally. On the 2d day patient regained consciousness and was much improved, the temperature falling to 100° F., and pulse to 98. His only complaint was severe headache. The first blood sulfadiazine level 24 hours after sulfonamide therapy was reported 5.6 mg. In spite of large doses of sulfadiazine, the blood concentration of the drug rose slowly and reached 9.9 mg., 48 hours after chemotherapy was inaugurated. Subsequent sulfadiazine levels were no higher than 13.1 mg. reported on the 5th day. Pains in the right elbow and wrist first appeared on the 6th day, and these joints were found to be swollen, tender, painful and hot. The temperature fluctuated between 101° and 102° F. The joints were treated conservatively and large doses of acetylsalicylic acid given with only slight relief. The sulfadiazine was continued and on the 7th day the left elbow and left wrist flared up similarly. Temperature persisted at 100.4° F. The left knee became painful and swollen on the 8th day. A sulfadiazine



level of 9.8 mg. and blood count of 15,150 white blood cells with a normal differential were reported on the 8th day. Joint symptoms persisted and, because of this, sulfadiazine was stopped on the 13th day. Within 36 hours after discontinuance of the drug, all joint manifestations subsided and on the 16th day remittance of the fever occurred.

**Comment.** The spectacular improvement in this patient after all drug therapy had ceased—the disappearance of joint symptomatology and subsidence of fever—made it plausible to attribute these effects to drug therapy. Since drug fever occurs infrequently with sulfadiazine, and joint manifestations are still rarer, the problem of differential diagnosis between drug idiosyncrasy and an occurrence of a new meningococcic focus localized in the joints was a difficult one.

The evidence for sulfadiazine arthritis was as follows: first, the appearance of migratory arthritis in spite of 6 days of massive sulfadiazine therapy; second, a falling white blood count; and third, the prompt and dramatic subsidence of fever and arthritis following discontinuance of the drug. Notwithstanding these symptoms it was thought best to err on the side of conservatism and to continue the administration of the drug, since it is well known that meningococcic infections are notorious in their recurrence after inadequate sulfonamide therapy.

FIG. 4.—INCIDENCE OF TOXIC MANIFESTATIONS OCCURRING IN 134 CASES OF MENINGOCOCCIC INFECTIONS

Sulfanilamide		Sulfadiazine	
Complication	Incidence	Complication	Incidence
Acidosis . . . . .	1	Drug fever . . . . .	3
Drug fever . . . . .	3	Arthritis . . . . .	2
Cyanosis . . . . .	4	Anuria . . . . .	1
Hemolytic anemia . . . . .	2	Oliguria . . . . .	4
Leukopenia . . . . .	0	Hematuria:	
		(a) Microscopic . . . . .	17
		(b) Gross . . . . .	11
		Renal colic . . . . .	6
Total cases . . . . .	*8	Total cases . . . . .	*36

\* Several cases had more than one complication.

It is noteworthy to add that the patient gave an additional history of having sustained a fall, injuring both elbows, both wrists and his left knee, 1 week prior to his present illness. This brought up the interesting question of whether previously traumatized joints are more prone to sulfadiazine arthritis than normal joints. In one other case in our series an arthritic complication of a previously injured joint occurred under sulfadiazine therapy. The second patient with joint involvement injured his right knee while playing football 4 years prior to his present illness, and a diagnosis of bursitis was made. During the course of his meningococcic infection and on the 6th day of sulfadiazine administration, this traumatized knee became red and swollen. The knee signs and symptoms promptly subsided when the sulfadiazine was stopped. No other joints were involved.

Another important point which this case aptly illustrates is the disproportion between administration of large doses of sulfadiazine and

low blood concentrations of the drug. The highest blood level recorded in this case was 13.1 mg. on the 5th day, with levels varying between 4.4 and 13.1 mg. Nevertheless, low blood sulfadiazine concentrations do not militate against clinical recovery. The exact rôle of blood sulfadiazine levels as a factor in clinical improvement and cure is difficult to assay. Our experience has taught us that certain cases with satisfactory blood levels have done poorly while other cases with low blood concentration have had a remarkably smooth course. Low blood sulfonamide levels are not inconsistent with complete clinical cure, and the explanation may lie in the delicate balance existing between the rate of absorption of the sulfa drug from the bowel and the rate of elimination from the kidneys. It must be assumed in this patient that the relatively low blood concentration was due to either slow absorption from the intestinal tract, rapid renal excretion, or to diffusion and storage of the drug in the tissues. Slow reabsorption from such a tissue reservoir might well explain the continued elevation of sulfadiazine blood levels after cessation of therapy, which has been observed in some instances.

**Summary.** We have described in this paper our clinical experiences in treating 134 consecutive cases of meningococcic infections with massive doses of sulfadiazine and sulfanilamide. The total mortality for the entire series, treated and untreated, was 9 cases (6.7%). In our treated cases there were 4 deaths (a mortality rate of 3.1%). Analysis of our fatalities showed: (a) 5 cases (3 treated and 2 untreated) clinically and on postmortem exhibited the Waterhouse-Friederichsen syndrome; (b) 4 cases of meningitis, 1 treated and 3 untreated, which were proven on postmortem examination.

Although the diagnosis in a certain number of our cases could not be verified bacteriologically, sufficient clinical evidence in conjunction with laboratory data confirmed the clinical impression of a meningococcic infection.

FIG. 5.—THE INCIDENCE OF TOXIC MANIFESTATIONS AS RELATED TO THE MODE OF SULFADIAZINE ADMINISTRATION

Critical analysis of sulfadiazine		No. of cases	Per cent
I. Number of cases of meningococcic infection . . . . .		134	
II. Number of cases receiving sulfadiazine . . . . .		128	
(a) Oral sulfadiazine . . . . .		82	64
(b) Intravenous and oral sulfadiazine . . . . .		46	36
III. Number of cases having sulfadiazine complications—all types regardless of mode of administration . . . . .		36	28
(a) Oral sulfadiazine complications . . . . .		23	28
(b) Intravenous and oral sulfadiazine . . . . .		13	28
IV. Number of cases having sulfadiazine renal complications—all types regardless of mode of administration . . . . .		31	24
(a) Renal complications—oral sulfadiazine . . . . .		19	23
(b) Renal complications—intravenous and oral sulfadiazine . . . . .		12	26

The incidence of toxic drug manifestations in our series was higher than that usually reported. It is felt that this was due to the massive doses of the sulfonamide utilized. In our series, sulfadiazine was responsible for toxic reactions in 36 cases (28% incidence). Of this

group, 31 cases (86%) had some form of renal complication, exclusive of renal colic (Fig. 5). One case of sulfadiazine anuria was briefly mentioned. Two cases of sulfadiazine arthritis were discussed and evidence presented substantiating this diagnosis, with one illustrative case history.

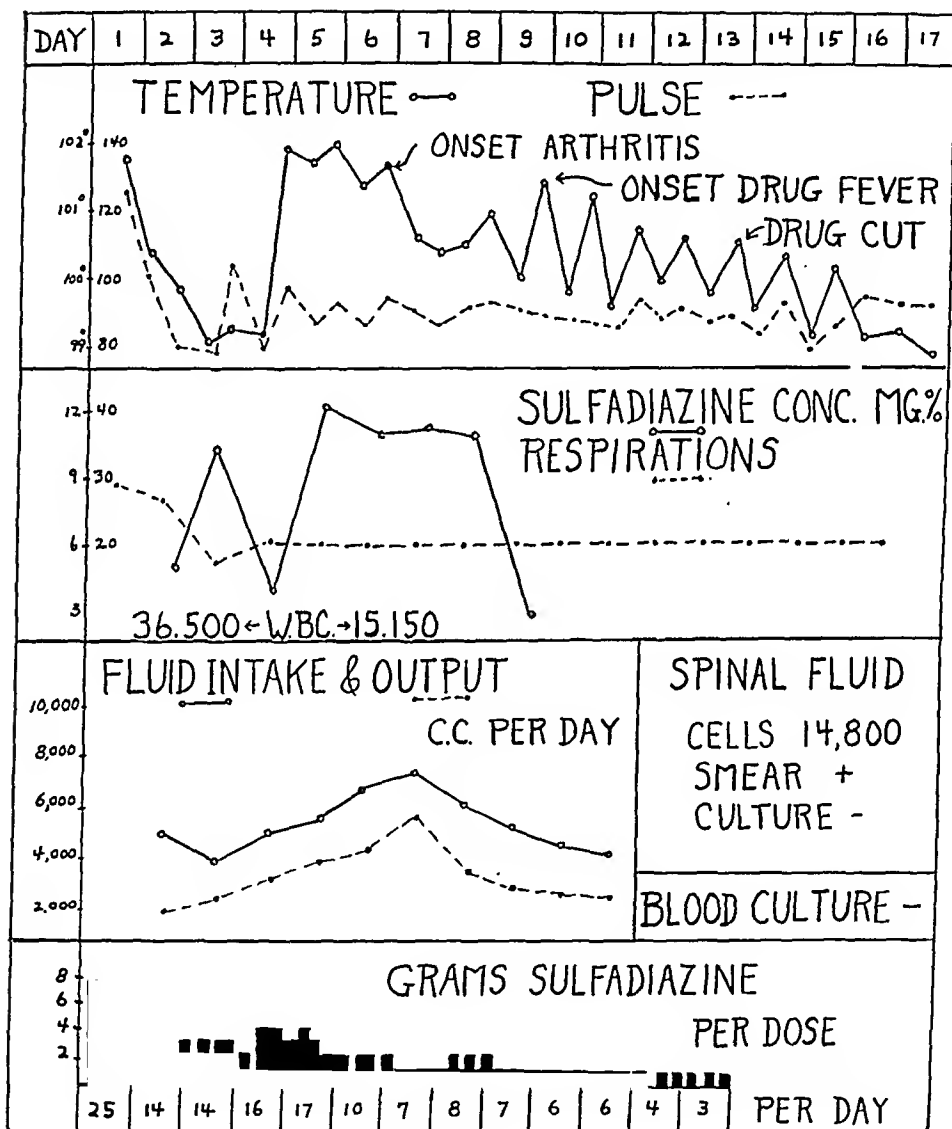


FIG. 6.—Case 1.

**Conclusions.** 1. From the results obtained in the treatment of 134 consecutive cases of meningococcic infections, we recommend the use of massive doses of sulfadiazine.

2. Our experience indicates that in the more seriously ill or moribund cases a satisfactory blood level is attained with difficulty with oral therapy. We feel that in these patients initial intravenous therapy should be used to complement oral medication.

3. The occurrence of 5 cases of the Waterhouse-Friederichsen syndrome in this group of patients indicates that it may be more frequent than hitherto reported in the literature.

4. An unusually high incidence of toxic manifestations due to sulfadiazine was encountered in our series of cases. However, our treated mortality rate of only 3.1% and no fatalities or residua which might be ascribed to the drug, justified the use of massive doses of sulfadiazine. Careful observation and supervision is absolutely essential in such massive therapy to prevent any undesirable consequences due to drug reactions.

5. Suitable evidence and data have been presented for the existence of sulfadiazine arthritis as a clinical entity.

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### IDIOPATHIC HYPOPROTHROMBINEMIA

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THE development of accurate methods for the determination of plasma prothrombin<sup>6a,12</sup> has led in recent years to a much clearer understanding of various hemorrhagic diseases so that 2 cases, formerly regarded as atypical hemophilia, have been shown to have suffered from an idiopathic hypoprothrombinemia.<sup>1,8</sup> We have lately had the opportunity to study a patient with an obscure hemorrhagic diathesis in whom the most constant and outstanding laboratory finding was hypoprothrombinemia, totally resistant to vitamin K therapy and lacking adequate explanation. This case would probably have been classed in the past as pseudohemophilia as his bleeding time was frequently prolonged, venous coagulation time always normal, platelet count normal and there was a history of abnormal bleeding in a sister. However, he shared the cardinal feature of both previously reported cases of idiopathic hypoprothrombinemia and there seems little doubt

that the deficiency of this factor was responsible for his hemorrhagic tendency.

**Case Report.** R. K., an 18 year old white male of Pennsylvania Dutch stock, was admitted to the medical wards of the Hospital of the University of Pennsylvania on 3/10/43 with the chief complaint of persistent bleeding from the gums and epistaxis of 3 months' duration.

The history began at the age of 4 when, after fracturing his nose in a fall, he developed profuse epistaxis which was controlled with difficulty. Thereafter, until the age of 10, nosebleeds, of varying degrees of severity, occurred about once a month. From the age of 10 years until his admission to this hospital epistaxis had become more frequent and severe, occurring 2 to 3 times per month, and in addition bleeding from the gum margins had first made its appearance. In October, 1939, he was admitted to the Easton Hospital, Easton, Pa., complaining of hematuria. Blood studies at this time revealed bleeding time  $1\frac{1}{2}$  minutes, coagulation time 3 minutes, prompt clot retraction and platelets 113,600.

Following severe epistaxis, the patient was admitted to the Allentown Hospital, Allentown, Pa., in January, 1943, where studies revealed a prolonged prothrombin time. He was then sent to this hospital for additional observation.

The past medical history was non-contributory except for the above-mentioned hemorrhagic tendency.

Family history, including investigation of father, mother, sibling, maternal and paternal grandparents, aunts, uncles and cousins revealed nothing of a contributory nature except for fatal bleeding in the patient's only sibling, a sister. The history of this girl was kindly made available by Dr. Leslie Freeman of Easton, Pa., and is as follows: B. K., a young white female, aged 4, was admitted to the Easton Hospital in June, 1931, with chief complaints of epistaxis and abdominal pain. The patient had been afflicted with nasal bleeding from the age of 3 years. Physical examination revealed an anemic child with tenderness around the umbilicus and hemorrhagic areas over the abdomen. Blood platelet count was normal on two occasions, bleeding time was 15 minutes and coagulation time 4 minutes. After several transfusions, the parents signed the child's release from the hospital. She was readmitted in October, 1932, with a history of profuse epistaxis of 3 weeks' duration. Examination revealed a palpable spleen. Laboratory studies showed severe anemia, normal platelet count and normal bleeding and clotting time. The patient died 14 hours after admission and the necropsy was reported as showing "splenic anemia."

Occupational and social history of R. K. was unessential, and there was no history of undue exposure to known toxic materials.

Physical examination on admission revealed an intelligent, alert but somewhat apprehensive young white male. Temperature was 98.3° F., pulse 73, blood pressure 100/60, height 66 inches, weight 104 pounds. The significant physical findings included evidence of recent bleeding in the nose, marked dental caries, a red, fissured tongue, congested and bleeding gums, palpable spleen which descended 1 to 2 cm. below the left costal margin with deep inspiration and bilateral sustained ankle clonus, not regarded as significant by a neurologic consultant. There was no evidence of jaundice and no petechiae or telangiectases could be found by careful examination, including nasoscopic study. The liver could not be felt and there was no evident lymphadenopathy. Ophthalmoscopic examination revealed normal fundi.

**Laboratory Studies.** *Blood:* R.B.C. 4,600,000, Hb. 77%, reticulocytes 7%, W.B.C. 7850, neutrophils 70%, lymphocytes 19%, monocytes 7%, eosinophils 4%. Red blood cell fragility tests showed hemolysis beginning at 0.45 and ending at 0.32 and on repetition comparable results were obtained. Erythrocyte sedimentation rate was 11 mm. per hour by the modified Rourke-Ernstene method. Figures for bleeding time, coagulation time, prothrombin time and blood platelet counts are shown in Table 1. Clot retraction was prompt and good and platelet morphology normal. Kolmer

and Klein tests were negative. Blood type was A and Rh+. *Urine:* Specific gravity was found from 1.012 to 1.028. There was no albumin or sugar and the urinary sediment was always quite normal. *Blood chemistry:* Blood urea nitrogen was 9 and 7 mg. per 100 ml., serum proteins 6.1 and 6 gm. per 100 ml. with the albumin 4.6 and globulin 1.4 gm. per 100 ml. giving an A/G ratio of 3.2. Serum calcium was 9.8 and 10.3 mg. per 100 ml., serum cholesterol 229 and 147 mg. per 100 ml., blood uric acid 4.8 and 3.7 mg. per 100 ml. and serum bilirubin less than 0.5 mg. per 100 ml. on two occasions. Plasma vitamin C level was 1.5 mg. per 100 ml. and plasma fibrinogen 331 mg. per 100 ml., both values being within normal limits. Glucose tolerance test showed a fasting level of 60,  $\frac{1}{2}$  hr. level 115, 1 hr. level 75 and a 2 hr. level of 90 mg. per 100 ml. *Liver function studies:* Cephalin-cholesterol flocculation test showed a 5 mm. flocculum in 48 hours. Hippuric acid excretion was 1.22 gm. in 1 hour and bromsulphalein test revealed 5% retention of dye after 30 minutes. *Roentgen ray* films of chest were normal. Examinations of the gastro-intestinal tract were normal, except for a roundworm seen on one occasion. Body section roentgenograph of the upper abdomen very successfully revealed a slightly enlarged spleen but normal sized liver. Roentgen ray of skull and urogram were within normal limits. Dental Roentgen rays showed numerous carious teeth. *Miscellaneous studies:* Two Gothlin indices as well as the usual tourniquet test were negative. Electrocardiogram was normal. Examination of bone marrow aspiration revealed normal inhabitants in the usual number. Urea clearance was 75% of average normal function. Microscopic examination of the capillaries of the nail bed revealed definitely abnormal and bizarre forms.

Because of the hypoprothrombinemia the patient was given several naphthoquinone derivatives by different routes. On 3/17/43, he was given over a 24 hour period a continuous venoclysis of 3000 cc. of 5% glucose to which was added 20 mg. of Synkavite (2-methyl 1, 4 naphthohydroquinone—diphosphoric acid ester tetrasodium salt). Before the start of the venoclysis the prothrombin time was 70 seconds and at the conclusion the figure was 80 seconds (normal control 16 seconds). From 4/4/43 to 5/7/43 the patient received Proklot (2-methyl 1, 4 naphthoquinone) mg. 1 p.o. with bile salts and Synkavite mg. 10 i.m. daily. As may be seen from Table 1, the plasma prothrombin level was not significantly affected. It was also clear that these preparations of vitamin K had no effect upon the bleeding. From 4/19/43 to 5/7/43, the patient was given Hesperiden mg. 250 t.i.d. without effect. On 5/12/43 administration of adrenal cortical extract was tried. Prothrombin time before administration was 64 seconds. Cortin (Upjohn), 20 cc., was given i.m. and 5 hours later prothrombin time was 58 seconds. Then an additional 10 cc. were administered, 5 cc. i.m. and 5 cc. i.v. Five hours after the second and 10 hours after the first dose, the prothrombin time was 54 seconds, a non-significant change. The only adequate hemostatic for this patient was whole blood and it was in every instance quite effective in stopping the bleeding. As little as 100 cc. of blood stopped hemorrhage within an hour and prevented bleeding for from 1 to 3 days. Administration of 500 cc. of blood usually inhibited bleeding for about 1 week.

Shortly after admission it was discovered that the patient was infested with *Ascaris lumbricoides*. Hexylresorcinol therapy was efficacious. The prothrombin levels were in no wise affected by the removal of the worms. During the hospital stay, 6 teeth were extracted and by means of frequent small transfusions, bleeding was kept to a minimum. Beef thrombin, applied locally to the socket failed to inhibit bleeding, an observation also made when it was applied to the bleeding gums.

Recently, it has been reported by Link *et al.*<sup>4</sup> and Rapoport *et al.*<sup>7</sup> that administration of salicylates may result in hypoprothrombinemia in experimental animals and man. However, in our patient both complete avoidance of salicylates (of which he had been getting an occasional 10 gr. dose) for a period of 3 weeks as well as administration of large doses for several days were without effect on his plasma prothrombin.

Lacking precedent, the question of future therapy and prognosis was difficult to answer. Temporarily a hospital job has been arranged for the boy so that transfusions may be made available as needed.

**Special Hematologic Studies.** Studies of the blood coagulability of the patient and his family are seen in Table 1. Our patient showed an invariably prolonged plasma prothrombin time by the Quick method,<sup>6b</sup> unaffected by vitamin K therapy or blood transfusion. The bleeding time was prolonged on several occasions but the coagulation time, clot retraction and platelet count were always within normal limits. The father had a slightly prolonged prothrombin time, probably of no significance, and normal bleeding time, coagulation time and platelet count. The mother's studies were quite normal. Studies done at the Easton Hospital in 1931 on the sister's blood showed increased bleeding time, normal coagulation time and platelet count. No prothrombin determination was done.

TABLE 1.—SOME BLOOD DATA IN THE K FAMILY

Date	Prothrombin time (Quick)* (sec.)	Bleeding time (Duke) (min.)	Coagulation time (Lee-White) (min.)	Platelet count (in thousands per c.mm.)
<i>Patient R. K.</i>				
3/11/43 . . . .	68	12	7	280
3/17/43 . . . .	70	..	10	234
3/18/43 . . . .	80			
3/22/43 . . . .	70	4	..	358
3/23/43 . . . .	79			
3/24/43 . . . .	..	6	7	
3/31/43 . . . .	92			
4/ 2/43 . . . .	80			
4/ 5/43 . . . .	60			
4/ 6/43 . . . .	..	1½	..	234
4/17/43 . . . .	85	2½		
4/20/43 . . . .	..	5½	10	254
4/21/43 . . . .	72			
4/28/43 . . . .	90			
5/17/43 . . . .	100	15	10	291
<i>Father of R. K.</i>				
4/18/43 . . . .	19	2	9	280
<i>Mother of R. K.</i>				
4/ 8/43 . . . .	16	1½	8	256
<i>Sister of R. K.†</i>				
5/12/31 . . . .	..	15	4	219
5/23/31 . . . .	..	..	..	297

\* Normal control—16 seconds.

† Studies done at Easton Hospital, Easton, Pa.

In order to check for the presence of antiprothrombin and antithrombin our patient's plasma was mixed with normal plasma in equal parts. The prothrombin time of the normal plasma was 16 seconds and that of the mixture was 18 seconds. This prolongation of the prothrombin time was exactly the same as that obtained after mixing the normal plasma with a prothrombin-free fluid such as 0.85% NaCl solution and was thought to demonstrate the absence of an excess of antiprothrombin or antithrombin.

To rule out the possibility that an excess of heparin was a factor, protamine was mixed with R. K.'s plasma in concentrations of 1/25, 1/50, and 1/75 mg. of protamine to 1 cc. of plasma. These mixtures caused prolongation of the prothrombin time but only to a degree to be expected from dilution with saline solution. In addition, the patient was given 40 mg. of protamine intravenously. Before injection, the prothrombin time was 92 seconds, and 30 minutes after injection it was 65 seconds. No further attempt was made to give the patient protamine because of the development of a marked sensitivity

to this protein. The significance of the finding is difficult to interpret but it may be stated that the normal coagulation time associated with marked hypoprothrombinemia speaks strongly against an excess of heparin as being the cause of the low plasma prothrombin and bleeding in this patient.

It was felt necessary to check the prothrombin along the lines suggested by Warner, Brinkhous and Smith.<sup>12</sup> Accordingly such a procedure was carried out by Miss Lillian Panzer on 5/5/43. After incubating the fibrinogen-free plasma of a normal control with thromboplastin and calcium for 90 seconds, a solution of fibrinogen was added and a clot formed in 14 seconds at a plasma dilution of 8 to 1 while R. K.'s plasma, treated in the same fashion required 48 seconds to clot in a dilution of only 2 to 1. The results were taken to mean that the prothrombin content of R. K.'s plasma was quantitatively low and that the fault was not merely in the conversion of prothrombin to thrombin.

Since the plasma fibrinogen of one of the reported cases of idiopathic hypoprothrombinemia was apparently abnormally resistant to the action of thrombin,<sup>8</sup> it was thought best to check R. K. for such an abnormality by the addition of fibrinogen preparations to his plasma. On 4/21/43 a solution of fibrinogen, freshly prepared from normal blood was mixed in equal parts with R. K.'s plasma. Before the addition of fibrinogen, the prothrombin time was 72 seconds while that of the mixture was 45 seconds. This was repeated on 4/28/43 and again the prothrombin time was reduced, this time from 90 to 65 seconds. A prothrombin determination done on the fibrinogen solution showed no clot in 900 seconds which indicated that the solution was practically free of prothrombin. An additional study involving the addition of deprothrombinized normal plasma, prepared by shaking with colloidal aluminum hydroxide, to R. K.'s plasma showed no reduction in the prothrombin time. This result was difficult to interpret. Nevertheless, it seemed that there was definitely suggestive evidence of some type of qualitative defect of R. K.'s plasma fibrinogen.

Another curious finding was that dilution of R. K.'s plasma with equal parts of saline only increased the prothrombin time from 79 to 94 seconds, definitely less than would be expected. This observation was also made in a previously reported case of idiopathic hypoprothrombinemia.<sup>5</sup>

**Discussion.** In 1941, Rhoads and Fitz-Hugh<sup>8</sup> reported the case of a young white male, previously diagnosed as atypical hemophilia, in whom the discovery of marked hypoprothrombinemia, resistant to the administration of vitamin K, required that the diagnosis be changed to idiopathic hypoprothrombinemia. The patient died as a result of central nervous system hemorrhage and examination of the liver revealed some atrophy of liver columns but the changes were considered too slight to have been the cause of the low plasma prothrombin. The case of Beard, quoted by Quick,<sup>1</sup> was similar in many respects to that of Rhoads and Fitz-Hugh but in spite of the fact that the bleeding continued in this case, the hypoprothrombinemia responded favorably to vitamin K therapy. Our patient had certain important features in common with the patient of Rhoads and Fitz-Hugh. Both had marked hypoprothrombinemia unresponsive to the administration of various preparations of vitamin K given by various routes. The platelet count of both was never low enough to account for abnormal bleeding. Hemorrhage in both had started early in life and their bleeding times were frequently prolonged. Special studies revealed that the plasma fibrinogen of both patients was qualitatively defective although this was shown less well in our patient. There were, however, definite differences between the 2 cases. The coagulation time of R. K. was



always normal, clot retraction good, bleeding usually from nose or gum margins and there was a familial history of bleeding in a female sibling. The patient of Rhoads and Fitz-Hugh, on the other hand, had a prolonged coagulation time, poor clot retraction, hemarthrosis was common and there was no familial history of abnormal bleeding. Despite these differences, it seems fair to reemphasize the fact that the bleeding in both patients was probably due to unexplained hypoprothrombinemia and that they, therefore, deserve to be classified in the same general group which as yet is not understood well enough to stand sub-classification.

Another significant point is that R. K., except for the hypoprothrombinemia, seemed to fit quite well into that rather ill-defined disease entity called pseudohemophilia, and in fact such would have probably been his diagnosis had not the prolonged prothrombin time been discovered. Increased but variable bleeding time, normal coagulation time and normal platelet count, found in R. K., are characteristically present in pseudohemophilia. Abnormal capillaries, seen in the nail-bed of our patient, have been shown by Macfarlane<sup>5</sup> to be present in pseudohemophilia. The type of bleeding, negative tourniquet test and even the slightly enlarged spleen are compatible with a diagnosis of pseudohemophilia. The bisexual hereditary and familial history, required for a definite diagnosis of this disease, is represented in our patient in part by a fatal hemorrhagic disease in the only sibling, a sister. A review of the literature reveals that those few cases of pseudohemophilia<sup>3,9,10,11</sup> in which plasma prothrombin was apparently determined by accurate methods, had normal or only slightly increased prothrombin times. There is, however, distinct laboratory and clinical variability among the cases of hemorrhagic diathesis which have been grouped together as pseudohemophilia so that this disease is still not sharply defined.<sup>2</sup> Since the plasma prothrombin has been studied by modern methods in only a few cases of this disease, it seems possible that prothrombin determinations on a large number of these patients might demonstrate a lack of this factor as the cause of bleeding in certain cases of pseudohemophilia.

The cause of the hypoprothrombinemia in our patient can be only speculative. There are 5 main situations in which a low prothrombin is found,<sup>6</sup> namely: deficiency of vitamin K; inadequate absorption of vitamin K from the gastro-intestinal tract; poor utilization of vitamin K by the liver; action of toxins (dicoumarin); and idiopathic. The first 2 situations were shown not to be present in our patient as there was no significant response of his plasma prothrombin to the parenteral or oral administration of vitamin K. If this patient's marked hypoprothrombinemia were due to poor hepatic utilization of vitamin K on the basis of liver damage, one might reasonably expect definitely abnormal results in some or all of the tests for liver function which was not the case with R. K. as studies of hepatic function, taken as a whole were not abnormal. Furthermore, liver size was normal and the patient did not present the clinical picture usually associated with liver disease of a degree required to give this severe and resistant

hypoprothrombinemia. Nevertheless, the poor sensitivity of the liver function tests in use today does not allow the complete elimination of liver damage as the cause for this patient's low plasma prothrombin. One could hypothesize, however, that R. K.'s primary fault was a specific inability of his liver to synthesize prothrombin, occurring in the presence of relatively normal liver function in other respects and possibly familial in character.

As to toxic causes of hypoprothrombinemia, it is interesting to note the similarity of R. K. to patients who have received overdoses of dicoumarin. However, we were never able to demonstrate any evidence that a toxin was influencing R. K.'s prothrombin and this seems further borne out by the fact that changes in diet and environment were without effect.

It appears then that the term idiopathic is needed to describe this case and that the cause of the hypoprothrombinemia cannot be definitely stated.

**Summary.** A case of apparent idiopathic hypoprothrombinemia has been presented which was in most respects comparable to 1 of the 2 previously reported cases of this condition.

An additional feature was the marked similarity of our patient's disease to pseudohemophilia.

This case presented many points of similarity to patients receiving overdoses of dicoumarin, but no adequate cause for the hypoprothrombinemia was demonstrated.

Since this paper was submitted for publication, another case has been reported which in most respects is similar to our case.<sup>2a</sup>

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# PROGRESS OF MEDICAL SCIENCE

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## OXYGEN POISONING\*

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**Introduction.** Lavoisier had no sooner discovered oxygen and its significance to life than experimenters began to subject animals to concentrations of oxygen greater than that in the air. Indeed, Lavoisier himself with Seguin<sup>118</sup> established the fact, since abundantly confirmed, that inhalation of 1 atmosphere of oxygen does not alter the oxidative metabolism. Early, administration of oxygen to the sick made a strong appeal to the imagination—both lay and professional. Rational oxygen therapy slowly evolved and was put upon a sound basis by the introduction of the arterial puncture—to determine hemoglobin unsaturation, and the oxygen chamber, tent, and mask—to assure efficient administration. The improved technical production of cheap, pure oxygen was, of course, an important contributing factor in this advance.

Two other fields of human activity were meanwhile increasing, the one old, the other new: namely, deep-sea diving and flying. Oxygen inhalation at greater than normal pressures assumed importance in these. Paul Bert, devoting years to the study of physiologic reactions in the first of these fields, embodied his findings in his classic, *La Pression Barometrique* (1878).<sup>40</sup> For the first time he revealed that oxygen plays a dual rôle. On the one hand—at low pressures—it sustains life; on the other—at high pressures—it kills. “One finds clearly demonstrated,” he said, “the apparently paradoxical result that under the influence of very high oxygenation

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of the blood, the tissues oxidize less, organic combustions diminish in energy, production of  $\text{CO}_2$ , excretion of urine, the destruction of sugar in the blood are diminished, and that as a result temperature falls." (Reviewers' translation.)

Thus began the subject of oxygen poisoning. Literature has accumulated; at first sight scanty, but upon search, surprisingly extensive. Various points of view were emphasized—deep-sea diving, aviation, micro-organisms, new growths, enzymes, blood gas transport, therapy. Some wrote a paper or two; others devoted years to the subject. In his Harvey Lecture, Behnke<sup>30</sup> enumerated the fruits of these labors: Increased knowledge of alterations in the gaseous environment of man, increased efficiency of the diver and caisson worker, clarification of puzzling phenomena of flight, and greater possibility of salvage work on sunken wrecks.

There are three types of situations involving possible oxygen poisoning: (1) At high altitudes, as in flying, where supplemental oxygen is given to counteract the insufficiency of the air. Since the ambient pressure is low, there need be no excess oxygen pressure;\* hence, as Armstrong stated,<sup>12</sup> "There have been no harmful effects from the use of oxygen in aviation except some of a relatively minor nature." (2) In deep-sea diving, in which toxic pressures of oxygen may be quickly reached. It is the dream of those interested that an understanding of the mechanism of oxygen poisoning will enable man to descend to depths limited by the strength of materials rather than his physiology. (3) In disease, particularly pulmonary and cardiac, producing insufficient saturation of blood hemoglobin, alleviated by oxygen administration. Poisoning may arise here if the safe dose is exceeded.

The first exposition of the subject was that of Bert. Subsequently Hill<sup>95</sup> devoted a portion of his book "Caisson Disease" to partial review and discussion of oxygen poisoning. Apparently there has been no exhaustive review. The authors present here a discussion of significant papers on the subject.

Bert, the discoverer of the phenomenon called it, according to the Hitchcock<sup>40</sup> translation, oxygen poisoning. By this is meant any variation from the normal structure or function attributable to the action of oxygen which produces deleterious effects.

**Symptomatology.** Excess oxygen produces characteristic symptoms and signs, the rapidity of onset and severity of which vary with the partial pressure of oxygen. While there seems to be general agreement that oxygen toxicity constitutes a clinical entity, there is less agreement on the incidence of specific symptoms, with the exception of those referable to the central nervous system.

1. *Nervous System.* Bert<sup>40</sup> studied the effects of increased air pressure on several animals, including birds, dogs and cold-blooded species. The outstanding symptom in all cases was convulsion, characterized by tetanus and opisthotonus, said to resemble strychnine poisoning. Later investigators described similar convulsions at various pressures in several species. Bornstein and Stroink<sup>48</sup> found clonic spasms developed in rats at 8 atmospheres of oxygen, Hill<sup>95</sup> and Shilling and Adams<sup>152</sup> described convulsions in several mammalian species at 2 to 5 atmospheres. Libbrecht and Massart<sup>120</sup> observed convulsions in mice at 4 atmospheres, and Hederer and Andre<sup>91</sup> found convulsions the presenting symptom in rabbits at from

\* All pressures of oxygen will be stated in terms of atmospheres absolute. Ordinarily, when administering oxygen, it is sufficient to indicate the concentration by stating the per cent. At higher pressures, however, such a designation becomes confusing.

0.8 to 12 atmospheres of oxygen. Behnke *et al.*<sup>36</sup> subjected dogs to 4 atmospheres of oxygen for 2 to 4 hours, and found that 2 out of 9 developed convulsions, which were described as respiratory in type. Later, Shaw, Behnke and Messer<sup>151</sup> stated that "the symptoms of acute oxygen poisoning" (at 4 atmospheres of oxygen) "which are most apparent are the changes in blood pressure" (see below) "and the convulsive seizures of the head and neck." Although generalized convulsions have not been as frequently observed in man as in other species, Behnke stated<sup>30</sup> that "essentially oxygen induces transient idiopathic epilepsy in the apparently normal individual." More specifically, Bornstein and Stroink,<sup>48</sup> while finding no symptoms in man at rest for 45 minutes in 2 atmospheres of oxygen, observed clonic spasms of the legs with hyperactive patellar reflexes when the subject was working on a bicycle. Behnke, Johnson, Poppen and Motley<sup>34</sup> studied the effect upon man of 1 to 4 atmospheres of oxygen, and described the symptoms as all referable to the nervous system; these included more or less generalized convulsions, syncope, and loss of coordination and attention. Thomson<sup>164</sup> observed 2 naval officers under 4 atmospheres of oxygen; 1 of these developed facial twitches in 16 minutes and the other, a tremor of the lips. When the second subject was returned to air at normal pressure, clonic seizures were followed by unconsciousness. The possibility that the symptoms in the second case were due to aëro-embolism cannot be excluded. (See section on Aëro-embolism.) Behnke, Forbes and Motley<sup>33</sup> studied the effects of oxygen at 4 atmospheres on men. They measured visual changes to be described later, but also observed dizziness, stupefaction and a "sense of impending collapse." Substitution of air by removal of the mask was followed by recovery and a sense of exhilaration. Becker-Freysang and Clamann<sup>27</sup> subjected themselves to 0.9 atmosphere of oxygen for 60 hours, and after the first 24 noticed paresthesias of the fingers. Behnke<sup>28</sup> described true epileptiform convulsions developing 45 minutes after subjection to 4 atmospheres of oxygen. In a review of observations pertinent to submarine salvage work, Jenkinson<sup>109</sup> gave as characteristic symptoms twitching of the head and neck, convulsions and paresthesias. Moody and Howard<sup>132</sup> described convulsions in a child with pneumonia who was treated for 6 days in an oxygen tent with a concentration presumed to be over 0.7 atmosphere although it was not measured in this case.

The non-specificity, or general nature of the central nervous system response to high oxygen is suggested by the observations of Bean and Rottschaefer<sup>25</sup> that the removal of the cortex, and even of the cerebrum in dogs had no appreciable effect upon the convulsions produced by 5 to 6 atmospheres of oxygen.

There has been only one piece of work done on the special senses, by Behnke, Forbes and Motley,<sup>33</sup> who found that 3 atmospheres of oxygen produced, among other effects, a reversible loss of visual acuity, contraction of the fields to the tubular type (reminiscent to the Reviewers, of that seen in advanced central nervous system lues) but with pupillary dilatation.

The mental effects of oxygen at high pressures were first described in detail by Hill and Phillips.<sup>101</sup> They associated personality types with the appearance of certain symptoms. For example, those divers showing overcontrol, suppressed fears and resentment of observation and direction, designated by the authors as the "philosophical type," were prone to show work failures, dizziness and claustrophobia at 7 to 8 atmospheres of air pressure (oxygen = 1.4 to 1.6 atmospheres). In contrast, the

"practical type" were relatively free of symptoms. Nitrogen narcosis as a contributing factor in these symptoms was not discussed by the authors. Previous to this, Binger and Faulkner<sup>42</sup> had found that rabbits and dogs became drowsy within 3 days, breathing 0.8 atmospheres of oxygen, although Paine, Lynn and Keys<sup>137</sup> later produced lethargy in dogs in 2 hours at 1 atmosphere of oxygen. Davidson,<sup>77</sup> working with 1 atmosphere of oxygen, found no effect upon reaction time in choice tests in 20 minutes, but Shilling and Willgrube,<sup>154</sup> in a highly quantitative report, showed a slowing in problem-solution, cross-out tests and light-to-touch reactions with increasing pressure. The amount of slowing was also related to the subject's mental ability. To eliminate possible confusion with nitrogen narcosis, they studied these effects with increasing time at a given pressure and with increasing experience, and found the mental slowing was reduced in each case. Haldane<sup>90</sup> distinguished the mental effects of high oxygen from those of high carbon dioxide and nitrogen in the period of recovery: in the former, there is marked terror, but in the latter two, there is calmness. Behnke<sup>30</sup> described "mental torpidity" as characteristic of oxygen poisoning. Although the effects of oxygen on the respiratory system will be separately considered, it should be mentioned here that Barach<sup>15</sup> found, in patients with a preëxisting chronic anoxia, that inhalation of 0.5 atmosphere of oxygen produced profound but reversible disturbances in mental functioning, consisting of deep sleep and stupor followed by coma and delirium. This finding supports Hill's statements<sup>95</sup> that coma and paralysis are frequent symptoms in animals.

*Respiratory Symptoms.* As the discussion on the disorders produced by high oxygen will demonstrate, the respiratory system is second only to the nervous system in its apparent susceptibility to the gas. The first symptom to be expected is dyspnea. While Hill<sup>95</sup> pointed out that the earlier authors, from the time of Lavoisier to 1912, described respiration under increased air pressure as variously increased and decreased in amplitude and frequency, there seems to have been little mention of dyspnea. Hill found this symptom dominant in several species of warm-blooded animals, and earlier in onset than the central nervous system symptoms. Binger, Faulkner and Moore<sup>42</sup> found that dyspnea and cyanosis were the dominant symptoms in rabbits and dogs exposed to 0.8 atmosphere of oxygen for longer than 3 days. (This is related to the pulmonary changes to be described later.) Most of the rats placed in 1 atmosphere of oxygen for 3 to 4 days by Boycott and Oakley<sup>49</sup> showed increasing dyspnea as the only symptom, and died apparently from dyspnea described as "explosive." Paine, Lynn and Keys<sup>137</sup> also found dyspnea the most important symptom in dogs placed in 0.9 to 1 atmosphere of oxygen; the time of development varied from 4 to 75 hours. Gesell<sup>88</sup> found essentially the same symptoms.

Passing from dyspnea to more general variations in respiratory effort, Achard and Leblanc<sup>2</sup> described the death of guinea pigs and rabbits in 0.8 atmosphere of oxygen as due to asphyxia following slowed and jerky respiration appearing in the second day. Smith, Heim, Thomson and Drinker<sup>158</sup> subjected rats to 4 atmospheres of air pressure (0.8 atmosphere of oxygen), and noted that on the 3rd day, the animals were acutely ill, showing hyperpnea and cyanosis. In connection with the convulsive seizures described by Shaw, Behnke and Messer above,<sup>151</sup> these authors further stated that the seizures are "essentially inspiratory convulsions and are not unlike those in certain types of asphyxia." The convulsive period was, in fact, "terminated by respiratory failure." Behnke<sup>28</sup> found

in healthy males after a 7-hour "tolerance period" in 1 atmosphere of oxygen, an increased respiratory depth. Finally, Paine, Keys and Lynn<sup>137</sup> described the death of dogs in 0.9 to 1 atmosphere of oxygen within 48 to 120 hours as occurring after a period of respiratory distress. Marshall and Rosenfeld<sup>126</sup> found in addition that the administration of oxygen will enhance the respiratory depression from various drugs to the point of apnea and respiratory failure. Although more important from a metabolic point of view, the measurement of respiratory minute volume also bears a relationship to respiratory symptoms. While Hill<sup>95</sup> could find little change under compressed air, Clark-Kennedy and Owen<sup>72</sup> reported a decrease in pulmonary ventilation when the oxygen was raised to only 0.26 atmosphere (far below the minimum concentration at which most authors report symptoms). On the other hand, Bean,<sup>17,18</sup> found an increased respiratory minute volume in dogs at 4 to 5 atmospheres of oxygen, and Bean and Haldi<sup>24</sup> confirmed this in dogs at 5 atmospheres, although there was no increase at 1 atmosphere. Bean and Rottschaefer<sup>25</sup> also found an increase at 5 to 6 atmospheres in decorticate and decerebrate dogs. In man, however, Belinke, Johnson, Poppen and Motley<sup>34</sup> found no change in minute volume at pressures from 1 to 4 atmospheres. In fact, "the irritative effect of oxygen on the lungs was noted in only one subject" (out of 10) "on a single occasion," although an increase in respiratory rate was observed. The single occasion was at low pressure (1 atmosphere). Schwab, Fine and Mixer<sup>148</sup> found no change in respiration in patients breathing 1 atmosphere of oxygen for 3 hours. Similarly, Becker-Freysang and Clamann<sup>27</sup> could detect no consistent change in respiration in themselves at 0.9 atmosphere of oxygen for 60 hours, although at the end of the experiment one of the authors developed bronchopneumonia, presumably a result of the high oxygen tension. Vital capacity was decreased in 1 subject and unchanged in the other. In dogs subjected to 1 atmosphere of oxygen for 6 minutes, Watt, Dumke and Comroe<sup>168</sup> found a transient diminution in respiratory minute volume of 11 to 13%. After elimination of respiratory chemoreceptors by the denervation of the carotid and aortic bodies, however, there was no change, and sometimes an increase. The authors concluded that since the chemoreceptors are continuously activated by the usual degree of oxygen unsaturation of the blood, the decrease of this saturation by high concentrations of oxygen eliminates this activation, resulting in diminution in respiratory minute volume.

Two general conclusions seem warranted from the evidence given above. The first is that respiratory symptoms are dominant in the action of oxygen at 0.8 to 2 atmospheres, while central nervous system symptoms are dominant above this level. This may perhaps be because at high pressures, death due to central nervous system symptoms precedes the possible development of pulmonary symptoms. The second conclusion is that in any case oxygen appears to be less irritating to the pulmonary system in man than in other species. However, conclusions with respect to man must be guarded since data are limited.

*Cardiovascular Symptoms.* Because of the comparative paucity of data, it is convenient to group together the heart rate in the intact organism, the blood pressure and the vasomotor signs of pallor and flushing. Bert observed<sup>40</sup> that under 3 to 4 atmospheres of air pressure (oxygen = 0.6 to 0.8 atmosphere), the pulse rate was lowered, and there appeared to be an increase in arterial tension, although this was not measured. Hill<sup>95</sup> confirmed the results obtained by other early investigators who found no

appreciable change in either pulse rate or blood pressure. Parkinson<sup>138</sup> observed a definite decrease in pulse rate when men breathed oxygen through masks at atmospheric pressure for 30 minutes. A similar observation by Dautrebande and Haldane<sup>76</sup> with an increase in the bradycardia as the pressure was raised to 2 atmospheres was explained by these authors as a possible protective mechanism against excessive changes in respiratory metabolism. Bean found a definite decrease in pulse rate in dogs subjected to 4 to 5 atmospheres of oxygen<sup>17,18</sup> which he compared to a similar result from the administration of carbon dioxide. Richards and Barach<sup>143</sup> kept 2 normal subjects in 0.5 atmosphere of oxygen for 7 days, and while there was no appreciable change in cardiac output, they noticed a fall in pulse rate. Shaw, Behnke and Messer<sup>151</sup> concluded from their experiments on dogs that the first sign of oxygen poisoning was a fall in blood pressure, a point which was disputed by Bean and Rottschaefer<sup>25</sup> who found that the blood pressure change was not consistent in dogs at 5 or 6 atmospheres. They also found that the bradycardia was dependent upon an intact vagus, a point later substantiated by Bean and Whitehorn.<sup>26</sup> On the other hand, when observing men in 3 and 4 atmospheres of oxygen, Behnke, Forbes and Motley<sup>33</sup> noticed that a rise in blood pressure accompanied the other signs of poisoning, which included pallor and an increase in pulse rate. Becker-Freysang and Clamann,<sup>27</sup> after 24 hours in 0.9 atmosphere of oxygen, found no marked change in their pulses, with the exception of an attack of paroxysmal tachycardia (which is not necessarily related to the oxygen); they made no measurements of blood pressure. Schwab, Fine and Mixer<sup>148</sup> observed a bradycardia of 60 for the first  $\frac{1}{2}$  to 1 hour in patients breathing 1 atmosphere of oxygen; there was no change in blood pressure or color in 3 hours. However, at 7 hours in 1 atmosphere, Behnke<sup>28</sup> found that healthy males showed a rise in pulse rate accompanied by pallor, which was replaced by flushing as the time was extended. This was explained as a possible sympathetic stimulation followed by parasympathetic, suggesting the action of a histamine-like substance.

In connection with this, Willmon and Behnke<sup>170</sup> found 1 man who when exposed repeatedly to 2.5 atmospheres of oxygen, developed an allergic response, characterized by dermatitis and generalized wheals which were relieved by a preparation of histaminase. However, no similar cases have been found in the literature.

Several less clearly defined symptoms of oxygen poisoning are described in man and animals. Malaise is described by Becker-Freysang and Clamann,<sup>27</sup> perhaps due to the concomitant development of pneumonia; anorexia is described by Binger, Faulkner and Moore,<sup>42</sup> Boycott and Oakley,<sup>49</sup> and Paine, Lynn and Keys;<sup>137</sup> nausea and vomiting were observed by Becker-Freysang and Clamann<sup>27</sup> and Behnke<sup>28</sup> who also found several subjects under oxygen at 1 atmosphere who complained of substernal distress.

Summarizing the cardiovascular changes, although there is not complete unanimity, the bulk of evidence shows that high tensions of oxygen are usually accompanied by a bradycardia which is apparently of vagal origin. As to blood pressure variations, there is neither sufficient agreement nor evidence to warrant a conclusion. Similarly, the observations are too few and scattered to allow a generalization about other symptoms, which appear to vary considerably.

**Pathologic Anatomy.** Since a review of the symptomatology seems to warrant the conclusion that at tensions of oxygen up to 2 atmospheres, the damage is chiefly pulmonary, and at higher pressures it is chiefly upon the central nervous system, one looks first for lesions in these 2 systems,



Noting that oxygen at a greater concentration than 0.6 atmosphere is toxic, Armstrong<sup>11</sup> stated, "The pathology in these cases indicates that the toxic action is restricted to the respiratory tract with congestion, edema, pneumonia and death occurring in that order." Behnke<sup>28</sup> stated that pure oxygen is a pulmonary irritant. Shilling and Adams<sup>152</sup> concluded, "the convulsions and pulmonary damage are separate, unrelated phenomena, both caused by the high tension of oxygen but acting in different manners." Unfortunately, there have been no exhaustive studies made of nervous system lesions following oxygen poisoning, and there is no evidence that any occurs. In fact, with the exception of non-specific changes in a few other organs to be mentioned later, pathologic changes appear to be limited to the lungs. Hill<sup>95</sup> credits L. Smith with the discovery that a slow increase of oxygen pressure causes congestion of the lungs, with the result that there is no "quick rise in oxygen tension in the blood, and so the convulsions fail to appear." Hill describes edema and exudate as the chief features of lung pathology. The first detailed studies, however, were made by Karsner<sup>112</sup> and by Karsner and Ash<sup>113</sup> of both gross and microscopic changes following exposure to oxygen at 0.8 to 1 atmosphere. The lungs of rabbits so treated showed congestion, edema, epithelial degeneration and desquamation, fibrin formation and finally pneumonia, as the time of exposure increased to 48 hours, and as the concentration of oxygen was raised from 0.5 to 1 atmosphere. Smith, Bennett, Heim, Thomson and Drinker<sup>157</sup> carried out a similar study with rats exposed to 4 atmospheres of air pressure (equivalent to 0.8 atmosphere of oxygen). They found progressive cellular hypertrophy and hyperplasia of the alveoli which persisted for months after return to normal pressure. These changes occurred in both young and old rats, but to a greater extent in the older ones. The final histologic structure resembled an exaggeration of that normally found in unexposed young rats. The younger animals and those that had been previously exposed showed an increased tolerance or adaptation to high pressures. The possible protective function of these structural changes was suggested by further investigation along the same lines by Smith, Heim, Thomson and Drinker,<sup>158</sup> who also found thickening and hyalinization of the walls of pulmonary arterioles and large arteries, with occasional thrombosis. The vessels were said to resemble those seen in chronic nephritis. Hederer and Andre<sup>91</sup> also found greater tolerance to oxygen in young than in older rabbits; however, rather than an increased tolerance with previous exposure, they found increased sensitivity. A similar sensitization was found by de Almeida.<sup>78</sup> Behnke *et al.*<sup>36</sup> demonstrated pulmonary pathology in part of a series of dogs exposed to 4 atmospheres of oxygen, but found the onset of convulsions the same as in those without such changes. The basic pulmonary changes consisting of edema, exudation and congestion were all reported in various species by Bornstein and Stroink,<sup>48</sup> Achard, Binet and Leblanc,<sup>1</sup> Achard, Leblanc and Binet,<sup>2</sup> Binger, Faulkner and Moore,<sup>42</sup> Pfesser,<sup>140</sup> Armstrong,<sup>11</sup> Orzechowski and Holzknecht,<sup>135</sup> Binet, Bochet and Bryskier,<sup>41</sup> Paine, Keys and Lynn,<sup>136</sup> and Paine, Lynn and Keys.<sup>137</sup> In addition, true hemorrhagic extravasation was observed in turtles exposed to 0.9 atmosphere of oxygen by Faulkner and Binger.<sup>82</sup> Other pulmonary signs of oxygen poisoning include massive pleural effusion, seen in rats exposed to 1 atmosphere by Boycott and Oakley,<sup>49</sup> pulmonary hemorrhage in several mammals, seen by Shilling and Adams<sup>152</sup> in pressures as high as 4 atmospheres of oxygen, and atelectasis at 4 atmospheres, found in dogs by Behnke *et al.*<sup>36</sup> The literature contains no reports of pathologic studies

of the lungs of man dying from the effects of high oxygen pressure, but there is some indirect evidence. When Becker-Freysang and Clamann<sup>27</sup> exposed themselves to 0.9 atmosphere of oxygen for 60 hours, one of them developed all the signs of bronchopneumonia at the end of the experiment, and required 10 days to recover. Jenkinson<sup>109</sup> drew upon his experience in submarine salvage work to state that high concentrations of oxygen at atmospheric pressure administered for several hours may bring on consolidation.

There is no evidence in the literature of lesions in the central nervous system.

There is a scattering of observations on the lesions in other organs following exposure to high oxygen tensions. Achard, Leblanc and Binet<sup>2</sup> described congestion in abdominal viscera, de Almeida<sup>78</sup> found atrophy of the testicles in rats, and Paine *et al.*<sup>136,137</sup> observed intense contraction of the spleen and distention of the stomach.

In conclusion, with the exception of minor effects upon abdominal viscera, the morphological changes caused by high pressures of oxygen are apparently limited to the lungs and pulmonary vessels.

**Blood.** 1. *Formed Elements and Hemoglobin.* Surprisingly, the first record found in the literature of a quantitative report on hemoglobin and red cell changes in the circulating blood under the influence of increased oxygen tensions was that of Campbell in 1926,<sup>59</sup> who observed a decrease of both in rabbits breathing 0.5 atmosphere of oxygen for 5 weeks; this change he considered a part of acclimatization. In contrast, Achard, Leblanc and Binet<sup>2</sup> found, in short exposures (0.8 atmosphere for 2 days), a marked increase of both red and white cells in rabbits and guinea pigs. In later papers, Campbell<sup>66</sup> reports a decrease in hemoglobin and red cells in several species of mammals in oxygen at about 1 atmosphere, and in rats at 6 atmospheres.<sup>57</sup> Izumiyami<sup>108</sup> likewise found a diminution in hemoglobin and red cells in 7 human subjects breathing 1 atmosphere of oxygen.

Boycott and Oakley,<sup>49</sup> in order to determine marrow activity, estimated the reticulocytes as well as the hemoglobin concentration in rats in 0.5 and 0.65 atmosphere of oxygen for 2 months. While there was some diminution in the hemoglobin concentration at 65% atmosphere, they concluded from the reticulocyte counts that, if anything, marrow activity was merely slowed down. Anthony,<sup>9</sup> and Anthony and Beudenkopf<sup>10</sup> made extensive measurements of human red cells and hemoglobin while the subjects were breathing oxygen through masks for 20 minutes and found an average drop in number of red cells of 7.8% and in hemoglobin of 3.3% with a corresponding increase in the color index. While breathing 0.9 atmosphere of oxygen, Becker-Freysang and Clamann<sup>27</sup> found a slight increase in red cells, and Paine, Keys and Lynn<sup>136</sup> showed a rise in hemoglobin concentration in dogs breathing 1 atmosphere. Similarly, Binet, Bochet and Bryskier<sup>41</sup> found, after an initial fall, a marked rise in red cells of guinea pigs, mice and pigeons in 0.7 to 1 atmosphere of oxygen.

The possibility that high pressures of oxygen alter blood hemoglobin apparently has not been investigated. Brooks,<sup>52</sup> however, reports a study on the relation of the rate of oxidation of hemoglobin and the partial pressure of oxygen. In the presence of certain oxidizing agents the rate is maximum to an oxygen pressure of 22 mm. The significance of this to oxygen poisoning is unknown.

Summarizing the changes in blood cells and hemoglobin, while the majority of observers with the most extensive data report a decrease in

red cells and hemoglobin, a minority report the opposite. Symptoms of pulmonary irritation or variations in the concentrations of oxygen studied do not give a consistent explanation for the difference. There are only 3 reports in the literature of determinations of white cells. Aehard, Leblanc and Binet<sup>2</sup> found them to be increased in guinea pigs and rabbits breathing 0.8 atmosphere of oxygen, and 1 of 2 human subjects studied by Becker-Freysang and Clamann<sup>27</sup> showed slight leukocytosis in 0.9 atmosphere. Behnke, Johnson, Poppen and Motley<sup>34</sup> found a slight leukocytosis without change in the differential from 2 hours exposure to 3 atmospheres of oxygen.

2. *Blood Chemistry.* Shilling, Thomson, Behnke, Shaw and Messer<sup>153</sup> determined a great many chemical blood and urinary constituents in animals exposed to high oxygen pressure, but found appreciable changes only in inorganic phosphate and sugar in the blood, which decreased. The drop in blood sugar confirmed a similar finding by Izumiyami<sup>108</sup> in man and animals. They also found a decrease in chlorides, serum albumin and viscosity, which returned rapidly to normal after removal from the oxygen. Although Shilling *et al.*<sup>153</sup> found no great change in non-protein nitrogen, Binet, Boelhet and Bryskier<sup>41</sup> found a rise in blood urea and uric acid in mice, guinea pigs and pigeons, and Paine, Lynn and Keys<sup>137</sup> found a 6 mg. per 100 cc. rise in non-protein nitrogen in dogs. Campbell<sup>63</sup> reports a significant increase in blood histamine in rats following exposure to 5 atmospheres of oxygen (0.5 to 1.3 gamma per cc.), the meaning of which is not apparent since he also found that injection of histamine had no effect on oxygen poisoning. This may be related, however, to Willmon and Behnke's finding<sup>170</sup> of an allergic response to oxygen in a man, relieved by histaminase (see section on Symptoms).

The finding of an increase in blood lactic acid by Bean and Haldi<sup>24</sup> in dogs subjected to oxygen pressures up to 5 atmospheres is a solitary observation of uncertain meaning.

Summarizing the changes in the blood, high oxygen pressures seem to produce a small but significant decrease in red cells and hemoglobin, and a slight leukocytosis. Various minor changes in blood chemistry occur, including an increase in histamine, but the significance of these is not clear.

**The Circulation and Peripheral Vessels.** Little work has been done on the actual measurement of cardiac output and blood flow, and the few observations recorded are limited to the vessels of the brain and retina. Pulse rate and blood pressure apparently tend to be lowered, although there is little consistency in the literature (see the section on Symptoms). Hill<sup>95</sup> confirmed a number of early authors that in compressed air, the circulation is unchanged. The only recent observation on cardiac output is that of Richards and Barach,<sup>143</sup> who could find no change in normal subjects breathing 0.5 atmosphere of oxygen for 1 week; this concentration, however, is usually considered outside the toxic range.

Tinel<sup>165</sup> found that high oxygen caused a constriction of the vessels of the brain. Wolff and Lennox<sup>172</sup> inserted a window over the pial artery of cats and observed a slight decrease in its diameter with 1 atmosphere of oxygen. While breathing a mixture of 90% oxygen and 10% carbon dioxide, the vessels were dilated and the animals showed only the symptoms of carbon dioxide poisoning. When the blood bicarbonate was artificially increased 39% the vessels were constricted, and when the carbon dioxide content was lowered by the intravenous injection of lactic acid, there was dilatation. Cobb and Fremont-Smith<sup>74</sup> studied the retinal circulation in man and found a change in size and color of the veins, but under a mixture

of 90 % oxygen and 10 % carbon dioxide, so their results cannot be interpreted as due predominantly to the oxygen. Cusick, Benson and Boothby<sup>75</sup> also studied retinal vessels. They found that 30 minutes exposure to 1 atmosphere of oxygen caused a 10 to 38 % decrease in the size of the vessels, the greatest change occurring in the veins. Finally, Bean<sup>17,18</sup> directly measured blood flow to the brain through an external cannula in dogs subjected to 4 and 5 atmospheres of oxygen, but could find no change.

The only conclusion that can be drawn from these data is that there appears to be a change in vascular diameter under the influence of high oxygen, but the extent of this change elsewhere than in the brain and its mechanism is not clear.

**Metabolism.** Measurements of the effect of oxygen pressures up to 1 atmosphere upon the total metabolism are naturally discussed separately from those made at higher pressures. The evidence available indicates that up to 1 atmosphere for relatively short periods oxygen has little or no effect. For example, Hill and Macleod<sup>99</sup> confirming the early work of Bert<sup>40</sup> found that 24 hour exposures to 1 atmosphere of oxygen slightly lowered the CO<sub>2</sub> output of mice. Benedict and Higgins,<sup>39</sup> measuring both CO<sub>2</sub> output and oxygen consumption reported 292 observations of the total metabolism on 6 normal men. They concluded from this abundant evidence that in men in the basal state "breathing 40 %, 60 %, and 90 % oxygen . . . there is no apparent difference between the metabolism as indicated by the gaseous exchange and the metabolism when breathing ordinary air."

As might be expected owing to additional physical solution, the initial uptake of oxygen by the intact animal rises with increased oxygen pressure. Hence the determination of metabolic oxygen is difficult until a steady state is reached. For this reason some observers have determined total metabolism by measurement of the CO<sub>2</sub> output only. However, Behnke, Johnson, Poppen and Motley,<sup>34</sup> as well as Benedict and Higgins<sup>39</sup> have excluded this factor of physical solution. For example, the former authors found in the case of 4 men breathing 1 atmosphere of oxygen that the oxygen consumption was constant for periods up to 4 hours, at levels not significantly different from the basal uptake as calculated from normal standards. But they did find an increased uptake during the first 20 minutes. They excluded increased physical solution and equilibrium with intestinal gases as the cause, but offered no further explanation.

No systematic studies on metabolism after prolonged (more than 24 hours) treatment with 1 atmosphere of oxygen appear to have been done. Since toxic symptoms usually supervene under these circumstances, changes should be expected. Stadie, Riggs and Haugaard<sup>161</sup> found that the respiration of slices of lung from dogs exposed to 1 atmosphere of oxygen for 48 hours was decreased by 30 % from the controls. Loss of weight has been reported by Binger, Faulkner, and Moore,<sup>42</sup> and Smith, Heim, Thomson, and Drinker.<sup>158</sup> However, the usual concomitant anorexia may have been as much responsible as metabolic changes.

Summarizing the metabolic changes, one may conclude that up to 1 atmosphere the inhalation of oxygen for 24 hours produces no significant change in the total metabolism. From the time of the discovery of oxygen by Lavoisier many systems of therapy have been built upon the assumption that the metabolism in normal and diseased states can be altered for the better by the inhalation of high concentrations of oxygen. Aside from the one rational use, *i. e.*, the alleviation of oxygen unsaturation of the blood in pulmonary and cardiac conditions, such conceptions still remain in the

realm of fancy. However, the possibility that lactic acid accumulation, oxygen debt, and so forth can be altered during short periods of vigorous exercise is real and has been discussed by Clark-Kennedy and Owen,<sup>72</sup> and Hill, Long and Lupton.<sup>94</sup>

When the oxygen pressure is raised appreciably above 1 atmosphere the situation is very different. Bert<sup>40</sup> did numerous experiments on rats, mice, sparrows and dogs using both air and oxygen to give partial pressures of oxygen ranging from 1 to 5 atmospheres. "In summary," he stated, "consumption of oxygen, production of carbonic acid and urica, breaking down of glucose in the blood, all chemical phenomena which can be measured easily, appear to be considerably slowed down by the action of oxygen under high tension. And as these are the phenomena which determine the production of heat, it is not surprising to see that the temperature of the animals drops considerably. Nor is it astonishing to see that death is the consequence of such depression in the intensity of the physico-chemical acts of nutrition." (Hitchcocks' translation.) By inference, Bert concluded that the action of oxygen in decreasing metabolic processes is due to its inhibitory effect upon the oxidative enzymes of the tissues (*v. infra*). Hill and Macleod<sup>99</sup> measuring the CO<sub>2</sub> output of mice, rats, and young rabbits, found a decrease at air pressures above 5 atmospheres and at oxygen pressures above 1 atmosphere. This, they concluded, is a constant concomitant of oxygen poisoning. Bean<sup>17,18</sup> also found a definite decrease of oxygen uptake in dogs under 5 atmospheres of oxygen.

**Blood Gas Equilibrium.** *Oxygen.* The relation between inspired air and blood gases is affected by variations in respiratory movement caused by central nervous system response to toxic levels of oxygen (convulsions), and by possible morphological changes in the alveoli. Hence interpretations of gas equilibria within the organism should be cautious. Nevertheless, Behnke *et al.*<sup>36</sup> have clearly demonstrated a prompt equilibrium between alveolar and pulmonary blood oxygen in dogs under 4 atmospheres of oxygen.

*Carbon Dioxide.* In the problem of blood and tissue carbon dioxide under high oxygen, the Reviewers believe that there has been in the literature an unfortunate and erroneous use of the word "retention." They define retention as an *accumulation* of carbon dioxide in blood or tissues owing either to impaired transportation or faulty elimination. They distinguish from this an increase in the partial pressure of *free* carbon dioxide, which does not necessarily mean retention as will be pointed out later. They further distinguish retention from *diminished metabolic formation*. Without these distinctions, discussion of the literature becomes confusing.

The question of carbon dioxide output under high oxygen must be discussed in the light of: (1) possible associated change of total metabolism, or (2) true carbon dioxide retention. While there are many experiments showing that carbon dioxide output is diminished under moderate oxygen pressures (0.8 to 2 atmospheres), concomitant studies on oxygen uptake have as a rule not been made, hence decrease in metabolism cannot be eliminated as the cause. Such experiments were reported by Bert,<sup>40</sup> Achard, Binet and Leblanc,<sup>1</sup> Achard, Leblanc and Binet,<sup>2</sup> and Becker-Freysang and Clamann.<sup>27</sup> On the other hand, Hill and his co-workers,<sup>95</sup> in experiments at these relatively low pressures, while finding similarly diminished carbon dioxide output, also noted an associated fall in body temperature sufficient to indicate a markedly lowered metabolism. In other words, there is no evidence of a true carbon dioxide retention under

these conditions; rather, decreased metabolism is a sufficient explanation. However, Hill suggested pulmonary damage as the cause. While this might easily explain an increase in the partial pressure of blood or tissue carbon dioxide as observed in other types of pulmonary pathology, it could only explain a temporary and not a persistent diminution of carbon dioxide elimination and even less, the increasing diminution which Hill observed. Furthermore, there is no evidence that true retention of carbon dioxide as we have defined it occurs even under pressures of oxygen greater than 2 atmospheres. In fact, Behnke *et al.*<sup>36</sup> demonstrated in dogs that exposure to 3.8 atmospheres does not significantly alter the *arterial*  $PCO_2$ , in sharp contrast to the increased  $PCO_2$  of the *venous* blood generally observed. In the Reviewers' opinion this is proof that there is no interference with carbon dioxide transportation or elimination. Finally, Behnke and Stephenson,<sup>37</sup> quoting their own unpublished experiments, were "unable to demonstrate any retardation in the elimination of carbon dioxide from the tissues" under high pressures of oxygen.

On the other hand, there is clear evidence that the *partial pressure* of carbon dioxide in the tissues is *increased* by high oxygen. Hill,<sup>96</sup> Gesell,<sup>88</sup> Campbell<sup>59-61</sup> and Behnke *et al.*<sup>36</sup> all found an elevation of  $PCO_2$  in tissues and *venous* blood. A consideration of the full evidence outlined above, particularly that of Behnke, has brought the Reviewers to the conclusion that this increased  $CO_2$  pressure is not due to retention as they have defined it. In their opinion the interesting and unique change in the acid-base balance of the blood known as the loss of the dual function of hemoglobin, first outlined by Gesell,<sup>88</sup> is a sufficient explanation. This is discussed more fully later.

Summarizing data on blood gas equilibrium, while there is evidence that high oxygen pressure produces a decrease in oxygen uptake, and *pari passu* in carbon dioxide output, these changes can be fully explained on the basis of lowered general metabolism. Changes in the partial pressure of carbon dioxide constitute a separate problem, accountable for, we believe, by Gesell's hypothesis.

**Influencing Factors.** *Gases.* At the outset, the distinction must be clearly drawn between the specific effects of high pressures of gases other than oxygen, and the effects which such gases produce on the toxicity of oxygen. In the first case, for example, the well-known narcotic effect of nitrogen at high pressures of air characterized by stupor, mental torpor, and so forth might be so great *per se* as to obscure more or less the effects of oxygen. Thus, Behnke, Thomson and Motley<sup>38</sup> contrasted the above-mentioned symptoms of narcosis produced by 3 to 10 atmospheres of air pressure with the convulsions, syncope, and so forth described by Behnke *et al.*<sup>34</sup> as accompanying 4 atmospheres of oxygen. They infer that under air pressure, the symptoms are largely those of nitrogen narcosis. They were confirmed in this conclusion by the experiments of Case and Haldane.<sup>69</sup> No experiments have been reported which would suggest any influence of nitrogen upon the toxicity of oxygen. These specific effects of gases other than oxygen do not fall within the scope of this review, but are mentioned in order to make the distinction clear.

Possible toxic action of contaminants of cylinder oxygen as causal agents has been excluded by the early work of Bert,<sup>40</sup> who found symptoms with cylinder oxygen or compressed air to be solely a function of the partial pressure of the oxygen. Binger, Faulkner and Moore,<sup>42</sup> in studies on rabbits, also excluded ozone as a possible cause of poisoning.

The most important gas to be considered in conjunction with oxygen is

carbon dioxide, because it figures largely in Gesell's discussion of the "dual function of hemoglobin" (*q.v.*). Gesell's hypothesis states that oxygen at high tensions increases the sensitivity of the animal to the administration of carbon dioxide owing to the broken coördination of the dual function of hemoglobin. This leads to acidosis which is further augmented by the administration of carbon dioxide, as he found to be the case in experiments on rats. The opposite explanation is offered by the observations of Shaw, Behnke and Messer<sup>151</sup> who produced subnormal levels of alveolar carbon dioxide tension by artificial hyperventilation, and supernormal levels by increasing the carbon dioxide in the mixture breathed, in dogs subjected to from 1 to 4 atmospheres of oxygen. Although the signs of oxygen poisoning appeared with subnormal alveolar carbon dioxide, they were hastened by an elevated carbon dioxide. The authors found, like Gesell, that elevated tensions of carbon dioxide which were not toxic at 1 atmosphere became associated with toxic symptoms in the presence of 4 atmospheres of oxygen; but, unlike Gesell, concluded that carbon dioxide might increase the toxicity of the oxygen or the sensitivity of the tissues to oxygen. However, it is obvious that no choice can be made between the two explanations on the basis of these experiments. In any case, since toxic symptoms of oxygen were found at subnormal levels of alveolar carbon dioxide; Shaw, Behnke and Messer concluded that carbon dioxide plays only a secondary rôle in oxygen poisoning. (For further discussion, see the sections on the Dual Function of Hemoglobin and Metabolism.)

In a more restricted experiment, discussed elsewhere with the question of circulation, Wolff and Lennox<sup>172</sup> found very slight arterial contraction under the influence of pure oxygen, and distinct dilatation when 10% carbon dioxide was added. In this respect, then, carbon dioxide appears to exert an influence exactly opposite to that of oxygen.

Hill<sup>96</sup> exposed monkeys, guinea pigs, rats, and goats to a mixture of 5% carbon dioxide in 1 atmosphere of oxygen, and then oxygen up to 5 atmospheres. The result was that the critical pressure of oxygen needed to produce convulsions within a given time, and the time necessary to produce them at a given oxygen pressure, were both distinctly lowered. He concluded that an "increase in carbon dioxide tension in the tissues is a factor in the production of convulsions which follow exposure to high pressures of oxygen." The same result was achieved by Massart<sup>127</sup> on mice, using 5% carbon dioxide in oxygen at 4 atmospheres, but his explanation is that respirations are increased (by the carbon dioxide) and hence there is a more rapid arrival at equilibrium with the oxygen. This explanation is untenable in view of the facts that respiratory rate is not materially affected by high air pressures (see the section on Symptoms), and that alveolar and blood oxygen are in equilibrium when pure oxygen is breathed (Behnke *et al.*<sup>36</sup>). The acceleration of oxygen toxicity by admixed carbon dioxide was also observed by Hederer and Andre.<sup>91</sup>

Libbrecht and Massart,<sup>121</sup> in an unconfirmed single report, stated that hydrogen had an antagonistic action to that of oxygen at high pressures. Mice, which had severe convulsions at 4 atmospheres of pure oxygen, when subjected to the same oxygen pressure plus 6 atmospheres of hydrogen, developed no convulsion but severe pulmonary symptoms only, although in the latter case the animals died sooner. This would indicate that the hydrogen acts as antagonist only to the effects of oxygen upon the nervous system as distinguished from those upon the pulmonary system.

*Other Factors.* Paul Bert<sup>40</sup> first observed, in conjunction with the general slowing of physiologic processes under compressed air, that body tempera-

ture was lowered. Hill<sup>95</sup> confirmed this, and made the further observation, which is difficult to explain in the light of later experiments, that he could protect animals to some extent by warming the pressure chamber. The first suggestion of the opposite effect of external temperature was given by Faulkner and Binger<sup>82</sup> who found that while turtles were more resistant than warm-blooded animals to 0.9 atmosphere of oxygen ordinarily, they became fully as susceptible to lung damage when the temperature was raised to 37.5° C. Perhaps this is owing to the increased metabolism of poikilothermous animals at elevated temperatures, a well-known phenomenon. Later, Campbell<sup>62-64</sup> made quantitative studies of the temperature effect on rats and found that a decrease in body temperature of about 10° C. increased the survival factor by about 10 times at pressures up to 6 atmospheres. The only temperature studies on men that have been done were concerned with the effects of carbon dioxide and nitrogen in compressed air, by Case and Haldane.<sup>69</sup>

One would expect that muscular exercise would alter the effects of high oxygen pressures, but no thorough investigations have been directed at this question. However, Bornstein and Stroink,<sup>48</sup> while finding no symptoms in themselves at rest in 45 minutes at 2 atmospheres, brought on cramps, clonic muscular spasms and hyperactivity of deep reflexes by exercises. Hill, Long and Lupton<sup>94</sup> studied the effect of exercise in man, but only at the non-toxic level of 0.5 atmosphere; they found a 10 to 50% increase in oxygen uptake, which was not due to increased saturation of the blood. It would be valuable to extend this investigation to higher concentrations of oxygen.

A number of drugs have been found to affect oxygen poisoning. The first investigation of drugs by Campbell<sup>69</sup> disclosed no effect by thyroid medication, although in later works,<sup>63,65,66</sup> he found that thyroxin and to a lesser degree certain other drugs, definitely enhanced the effect of oxygen on rats. In substantiation, he also found that the survival rate was increased following either thyroidectomy or hypophysectomy (to remove the thyrotropic effect of the posterior pituitary). He found that histamine had no effect (see section on Circulating Blood), but that oxygen poisoning was increased by dinitrophenol, ac-tetrahydro-beta-naphthylamine, adrenalin, extract of the posterior lobe of the pituitary, insulin, eserine and atropine. Marshall and Rosenfeld<sup>126</sup> found that oxygen increased the depression of respiration caused by anesthetics (including barbiturates and morphine, but not chlorbutanol, urethane, paraldehyde or alcohol) apparently by removing otherwise effective anoxemic stimuli which act through the sino-aortic mechanism. On the other hand, addition of carbon dioxide to the oxygen failed to prevent this enhanced depression, which suggests an effect due to a specific action of oxygen. A conflicting observation was made by Hederer and Andre<sup>91</sup> that barbiturates retarded the convulsions produced by oxygen, but as might be expected, strychnine enhanced them.

Finally, Campbell<sup>62</sup> and de Almeida<sup>78,79</sup> both found a marked increase in resistance to oxygen in rats that had been starved, and Smith *et al.*<sup>158</sup> found that the tolerance of rats was decreased late in pregnancy.

In summary, there are several factors which influence oxygen poisoning. Carbon dioxide clearly exerts an influence, apparently secondary, either by enhancing the effect of oxygen, or by contributing an independent effect enhanced by the oxygen. There is some evidence that it counteracts peripheral vascular contraction by oxygen but to what extent the latter occurs is not clear. Hydrogen appears to retard the effects of high



oxygen pressures, but there is no evidence as to an effect by nitrogen. Oxygen poisoning has been well shown to be diminished by a lowered body temperature, and *vice versa*, as well as by starvation, but to be augmented by several drugs, notably thyroxin and certain respiratory depressants.

**Tolerance, Adaptation and Oxygen Therapy.** Any discussion of oxygen poisoning naturally requires consideration of the maximum non-deleterious dose of oxygen at various pressures above the normal of 0.2 atmosphere. The observations in the case of man are more complete and consistent than those for other species, and will be considered first because of their importance.

The lowest concentration of oxygen at which physiologic changes have been noticed was reported as 45% by Richards and Barach,<sup>143</sup> who found that 2 normal subjects kept at this level for a week showed a fall in pulse rate and a slight rise in total carbon dioxide content of the blood. There were no changes in respiratory metabolism, cardiac output or excretion of electrolytes and water. Patients with cardiac insufficiency, however, showed good therapeutic response to oxygen at 0.4 to 0.5 atmosphere for periods up to 7 months without demonstrable signs of oxygen poisoning.<sup>144</sup> Binet *et al.*<sup>41</sup> described some minor physiologic changes in rabbits at 0.6 atmosphere. With these exceptions, the maximum oxygen concentration that can be inhaled by man and other warm-blooded species indefinitely without harm was given as approximately 0.6 atmosphere by Barach,<sup>14</sup> Boycott and Oakley,<sup>50</sup> Binet *et al.*,<sup>41</sup> Behnke and Shaw,<sup>35</sup> Becker-Freysang and Clamann<sup>27</sup> and Trusler and Meiks.<sup>166</sup>

Recently developed methods have made possible the use of oxygen at nearly 1 atmosphere for certain purposes, as described for example by Boothby<sup>46</sup> and Boothby, Mayo and Lovelace.<sup>47</sup> Behnke *et al.*<sup>34</sup> described symptoms occurring in 0.96 atmosphere oxygen at various times up to about 4 hours, which they gave as the limit of safe exposure. The important observation of Marshall and Rosenfeld<sup>126</sup> was discussed above, that under certain conditions of respiratory depression by anesthetics, the administration of oxygen will further depress respiration. While Anthony<sup>9</sup> and Anthony and Beudenkopf<sup>10</sup> describe a fall in the number of blood cells and hemoglobin produced by inhalation of approximately 1 atmosphere of oxygen for periods as short as 15 minutes, it is doubtful whether this can be considered a toxic effect, since it is reversible and was not reported as accompanied by symptoms. Fine, Hermanson and Frehling<sup>83</sup> reported no toxic symptoms from the use of 0.95 atmosphere of oxygen when the patients were taken out of the tents at least  $\frac{1}{2}$  hour out of every 4 to 8 hours. Schwartz and Malikiosis<sup>149</sup> reported the sudden development of clonic spasms and collapse in subjects who were given 1 atmosphere of oxygen following a period of breathing air at diminished pressure. Boothby<sup>46</sup> concluded that oxygen at 1 atmosphere may be administered for 24 hours, and Boothby, Mayo and Lovelace<sup>47</sup> reported the administration of 1 atmosphere of oxygen to 800 patients with no evidence of the development of pulmonary irritation in less than 48 hours, and none in several days with intermittent administration. Whether in all cases these patients were inhaling 1 atmosphere of oxygen is uncertain since the report gives no analyses of circumambient oxygen. Furthermore, the important factor of intermittence for feeding, medication, and so forth was not included in the data. The authors recommend, in conclusion, that 1 atmosphere of oxygen may be given for 36 to 40 hours, but then must be reduced to 0.6 atmosphere. Becker-Freysang and Clamann<sup>27</sup> felt no symptoms for 24 hours in 0.9 atmosphere of oxygen. Behnke,<sup>28</sup> on the other hand,

found that healthy males show a slowing of the pulse rate and pallor in 7 hours at 1 atmosphere, and beyond this time, an increase in respiratory depth with nausea and flushing. In a later paper, Behnke<sup>30</sup> stated that 1 atmosphere can in most cases be inhaled for 17 hours without injury, although some subjects have complained of substernal distress in 7 hours, and 1 subject developed an allergic response. Moody and Howard,<sup>132</sup> using an oxygen tent, observed, as previously mentioned, convulsions in a child, who spent 6 days intermittently in an atmosphere which, although not measured, was assumed to be in excess of 0.7 atmosphere of oxygen. The child, 2 years old, had pneumonia and had given a good response to sulfonamide therapy with the exception that cyanosis had persisted. The convulsions were relieved by removal from the tent, recurred on re-entrance, and were again relieved on removal. For this reason, and because of the absence of any other abnormal findings including fever, they were attributed to the oxygen.

The conservative conclusion by the great majority of those experienced in oxygen therapy is that 0.6 atmosphere of oxygen is relatively safe for an indefinite period, and 1 atmosphere for about 24 hours, with due consideration for the factors discussed elsewhere. Ruff and Strughold, quoted by Becker-Freysang and Clamann,<sup>27</sup> described what they called the "paradoxical oxygen effect." Subjects breathing oxygen at 1 atmosphere for a period of time, when transferred to air at somewhat diminished pressure, developed clonic spasms and collapse. This phenomenon has been observed in interceptor pilots who breathe oxygen at ground level for purposes of de-nitrogenation and then ascend quickly to high altitudes. It probably is not related to the problem of oxygen poisoning, but is perhaps explainable in the light of the observations of Watt, Dumke and Comroe,<sup>168</sup> discussed in the section on Respiratory Symptoms.

Young animals appear to be relatively resistant to the toxic action of high pressures of oxygen.<sup>91,157</sup> For example, Smith, Bennett, Heim, Thomson and Drinker<sup>157</sup> found that young rats were not so readily poisoned by exposures to 0.8 to 1 atmosphere of oxygen as were older ones, a fact which they were inclined to attribute to morphologic differences in pulmonary structure. This increased tolerance has also been observed in the human by Chapple.\* Employing a special incubator<sup>69a</sup> which permitted treatment without intermittence at oxygen pressures never less than 0.85 atmosphere, he has treated premature infants weighing less than 3 lbs. for periods frequently as long as 3 weeks. During treatment the humidity is maintained close to 100%. Under these circumstances, he observed no poisonous action of oxygen whatever, and is convinced that this form of oxygen therapy is of great value in this type of case.

It has been suggested that part of the deleterious action of oxygen especially upon the lungs may be due to its dryness when administered. No papers discussing the possible relation of humidity to oxygen poisoning have been found.

The few conflicting reports concerning adaptation are mentioned in the discussion of morphology and circulating blood, and allow only the conclusion that if adaptation occurs, it is due to the protective action of pulmonary pathology, and possibly, as pointed out by Campbell,<sup>60</sup> to the decrease in hemoglobin and red cells (*v. supra*).

In connection with the use of oxygen at pressures about 1 atmosphere,

\* Dr. Charles C. Chapple, of the Department of Pediatrics, University of Pennsylvania, has courteously permitted us to quote his work in advance of its publication in this Journal.

for example in preliminary de-nitrogenation of divers, Behnke and Shaw<sup>35</sup> have emphasized the importance of a thorough knowledge of tolerance levels. In view of the scattered nature and inconsistency of the observations at higher pressures, particularly in animals, the summary given in Table 1 of tolerance levels found by various investigators will serve as useful a purpose as an extended discussion of all the reports. As was pointed out earlier, the safe time limit can be seen to drop with increasing rapidity as the oxygen pressure is raised above 1 atmosphere. One explanation for this phenomenon, which supposes a protective effect at low pressures by pulmonary pathology, is discussed in the section on Pathology. It can also be seen by inspection of the table that there is a marked variation in tolerance to oxygen among different species. For example, while most mammals have almost instantaneous convulsions at 5 atmospheres, Cleveland's observations<sup>73</sup> on cold-blooded vertebrates show a markedly greater tolerance of these species.

TABLE 1.—A COMPARISON OF THE LEVELS OF OXYGEN POISONOUS TO VARIOUS SPECIES, FROM AVERAGE FIGURES IN THE LITERATURE

Oxygen tension (atmospheres)	Animal species	Lethal time (hrs.)	Asymptomatic time limit for man	Reference numbers
0.45	.....	...	7 days	95, 143
0.6	.....	...	Indefinite	14, 27, 35, 41
0.7+	.....	...	6 days	49, 132, 166
0.8	Rabbit	192	...	14, 42
0.85 to 1	Premature infants	...	> 3 weeks	Chapple (personal communication)
0.89 to 0.9	Mouse	36	24 hours	27, 140
	Dog	120	...	136, 137
0.95 to 0.96	Rat	144	...	34, 57, 154
	Cat	72	...	136, 137, 140
	Dog	108		
0.99 to 1	Dog	48	7 to 40 hr.	28, 30, 34, 46, 47, 136, 137, 140
2.0	Mammals	4	0.75 to 3 hr.	34, 48, 96
3.0	.....	...	0.50 to 2	34, 96, 109
3.5	Frogs	65	...	73
	Salamanders and goldfish	50 to 60	...	73
4.0	Mammals	0.48	0.20 to 0.70	34, 96, 164
5.0	Mammals	0 to 2 (rats)	...	48, 96
7.0	.....	...	0.10	90
8.0	Rats	0	...	48
9.0	.....	...	0.05	109
50 0	Rats, mice, etc.	0	...	99

One may conclude that the maximum non-deleterious dose of oxygen which can be used for indefinite periods appears to be 0.6 atmosphere, while for 1 atmosphere the time limit is 24 hours. Chapple's experience (*v. supra*) would appear to exclude premature infants from these limitations. The evidence is not sufficient to say that adaptation occurs to an important degree. Tolerance to high oxygen decreases rapidly with increasing pressures above 1 atmosphere, and is in general lowest for mammals and greatest for cold-blooded species.

**Oxygen Aero-embolism From Rapid Decompression.** There is a sole report in the literature: Hill,<sup>95</sup> in experiments on toads, rats and guinea pigs, showed the formation of bubbles in the heart and other organs following rapid decompression from high pressures of pure oxygen, but the

pressures used were very high, namely, 15 to 20 atmospheres. It is probable that decompression from lower pressures (2 to 5 atmospheres) would not be accompanied by aero-embolism, because oxygen, unlike nitrogen, is rapidly used for metabolic purposes and therefore would most probably not persist, to form gas emboli.

**Isolated Surviving Tissue.** The experimental work in this category is limited. Bert (1878)<sup>40</sup> reported that the oxygen uptake and CO<sub>2</sub> output of strips of beef muscle, when subjected to high oxygen, was diminished compared to controls.

Most of our later knowledge of this phase of the subject is due to the excellent series of papers by Bean and his associates. Bean and Bohr<sup>23</sup> found in the case of smooth muscle (duodenal and pyloric sphincter muscle of rabbits) subjected to 6 atmospheres of oxygen, a definite pattern of low peristalsis and spasm similar to that produced by HCN but different from that by atropine. He concluded that this was owing to a direct action of oxygen upon the enzymatic systems of the tissues resulting in low oxygen utilization, a condition which he called "hyperoxic anoxia." The effect was reversible only after comparatively short exposure to relatively low pressures; no reversibility occurred after exposure to high pressures. With radial beef iris muscle, Bean and Bohr<sup>22</sup> observed a decrease of tonus when the muscle was subjected to 4.8 atmospheres of oxygen. Controls in air at the same pressure were unaffected. Recovery of normal tone followed removal from the high pressure. Atropinization did not change the effect of high oxygen. The authors concluded that the pupillary dilatation observed at high pressures of oxygen is not necessarily dependent on central nervous or hematogenous connections. Studying mammalian smooth muscle (rabbit duodenal strips) Bohr and Bean<sup>44</sup> found a decrease of tonus after 2.5 hours at 6 atmospheres of oxygen. Reversal to normal occurred if the high oxygen period was relatively short, but otherwise a periodic tonic spasm supervened. They stated that the action is not due to an epinephrine-like substance at the myoneural junction. Bohr and Bean<sup>43</sup> studied the action of the isolated frog heart in 5 atmospheres of oxygen. They found an initial increase followed by a delayed slight decrease in the strength of the beat. Later there was a loss of automaticity, but irritability remained even after the beat had ceased. Recovery after decompression was observed if the experiment was terminated before the complete cessation of cardiac activity.

The same authors,<sup>21</sup> using isolated striated muscle of the frog, found after exposure for 1.5 to 5 hours to oxygen at 5 atmospheres an initial increase of contraction followed, however, by a marked decrease in response to electrical stimulation of muscle or nerve compared to controls in air at elevated pressures. Usually no recovery was observed after 2 exposures. Their data led them to believe that the effect is mainly if not all upon the muscle rather than the nerve fiber or the myoneural junction. Hill and Macleod<sup>99</sup> found that the thin sartorius muscle of the frog, after 1 hour exposure to 50 atmospheres of oxygen, showed a greatly diminished height of contraction and a prolonged latent period. The gastrocnemius muscle, however, appeared relatively little affected.

Bean and Bohr,<sup>21</sup> in discussing the problem of oxygen poisoning, laid considerable stress upon the loss of the dual function of hemoglobin as the main cause of tissue dysfunction. They attributed to the "accumulated carbon dioxide and acidity resulting from inadequate reduction of hemoglobin a more significant etiologic factor in the early signs and later events of oxygen poisoning" than the "direct" toxic action of high oxygen upon

the enzyme systems involved in metabolic processes. This aspect of the subject is discussed more fully later.

Hill and Macleod<sup>99</sup> observed that a frog's heart continued to beat after 1 hour's exposure to an oxygen pressure of 50 atmospheres. Cold-blooded animals are known to be relatively resistant to oxygen poisoning, nevertheless this is an extraordinarily high pressure of oxygen to be survived by tissues.

Burrows<sup>54</sup> found in the case of tissues from chick embryos that the rate of growth is slightly more rapid in an atmosphere of pure oxygen, but the total growth is no greater than at lower partial pressures. He did not study the effect of pressures greater than 1 atmosphere.

Summarizing the only evidence available on isolated surviving tissue, it shows a distinct decrease in the function of smooth and striated muscle, but no apparent effect on myoneural junctions. Other tissues have not yet been sufficiently studied to warrant a conclusion.

**Neoplasia.** A brief chapter on this subject has accumulated in the literature. Fischer and Andersen<sup>84</sup> reported that under increased oxygen pressure sarcoma cells were killed more quickly than normal tissue cells in artificial culture media. Their data, however, are not convincing. Later, Andersen and Demuth,<sup>8</sup> experimenting on 500 mice, reported that in some animals pre-treated intravenously with copper or selenium (having tumor affinity and possibly catalytic action) and kept in 1.5 to 2 atmospheres of oxygen for 24 hours, transplanted carcinomata could be made to disappear completely. Oxygen or the metal alone did not give the effect. Occasionally permanent recovery followed, but in most cases the tumor reappeared from incompletely necrotized tissue. Still later, Fischer and Andersen<sup>85</sup> again found with transplanted tumor in mice that treatment for 24 hours with 1 atmosphere of oxygen resulted in the disappearance of the tumor. Better results were obtained when the oxygen treatment was combined with copper or selenium injections. However, these results were obtained only if the treatment was given during the first week after the transplantation and they surmised that similar therapeutic success is doubtful in the case of humans. De Almeida,<sup>79</sup> using starved rats to increase oxygen resistance, found that sarcoma (fusiform cell type of Ruffo) could be made to disappear following treatment with oxygen at 1 to 8 atmospheres for 24 hours to 20 minutes. His best results were obtained with 6 atmospheres of oxygen for 2 hours. The tumors were completely destroyed by a single treatment; 10 days later they were red, impregnated with blood, and microscopically none of the tumor tissue was preserved. De Almeida expressed intentions of building a chamber for the treatment of human cases to be reported on later. We have been unable to find further reference to such work. Campbell<sup>68</sup> subjected de Almeida's work to close scrutiny. Using Bashford's mouse carcinoma (No. 63), Twort's mouse tumor, or Walker rat tumor, he was unable to demonstrate any effect upon the histology after treatment of the animals with 4 to 5 atmospheres of oxygen for 1 hour. Nor was any effect found on spontaneous mouse mammary carcinoma.

The possibility, then, that high oxygen pressures might differentially injure or kill malignant cells either in culture or *in situ* remains unsettled in view of these interesting but conflicting reports.

**Plants and Microorganisms.** Scattered observations indicate a toxic action of high oxygen pressure upon forms of life other than mammalian. Bean,<sup>19,20</sup> for example, observed that the growth of pneumococcus is a function of the oxygen pressure, being maximum at about 1.2 atmospheres

and then declining, to cease at a pressure of about 2 atmospheres. The organisms could be killed by exposures to 5.8 atmospheres for 1 to 2 hours.

Boothby<sup>46</sup> discussed the possibility of the bactericidal action of oxygen at 1 atmosphere in cases of gas gangrene and tetanus.

Karsner, Brittingham and Richardson<sup>114</sup> found that the growth of most of the common pathogenic bacteria is inhibited by 1 atmosphere of oxygen. The pneumococcus was an exception.

Novy and Soule,<sup>134</sup> studying the tubercle bacillus, observed excellent growth at 0.4 to 0.5 atmosphere of oxygen but reported definite inhibition at 0.8 to 1 atmosphere.

Rahn and Richardson<sup>142</sup> studied the growth of *Streptococcus lacticus*, *Pseudomonas fluorescens* and *Bacillus subtilis* under high oxygen pressures. The first was greatly inhibited by oxygen at 1 atmosphere. The others were uninfluenced.

Thaysen<sup>163</sup> reported that the growth of a number of common organisms was greatly retarded at 10 atmospheres of oxygen. The effect was dependent upon the temperature, being lethal if the temperature was raised slightly above the optimum for growth. This observation is similar to those on higher organisms, discussed elsewhere.

Massart<sup>128</sup> reported that the respiration of yeast was decreased by exposures to 3 to 5 atmospheres for short periods of time (15 to 60 minutes). He attributed this to a partially irreversible oxidation of cytochrome which in the oxidized state, he assumed, is without respiratory function. Albaum, Kaiser, and Eichel<sup>6</sup> showed that oat grains soaked for 24 to 48 hours in water saturated with oxygen at 1 atmosphere manifested inhibited growth. Albaum, Donelly and Korkes<sup>5</sup> found a similar effect with oat seedlings associated with a lowered catalase and endogenous dehydrogenase activity. Cleveland,<sup>73</sup> in an interesting paper, reported that intestinal protozoa infesting termites, earthworms, cockroaches, frogs, salamanders and goldfish, were killed *in situ* by 3.5 atmospheres of oxygen without injury to the hosts. However, intestinal trichomonas of rats or man were not killed *in vitro* by such pressures.

Summarizing the evidence on microorganisms, high oxygen causes toxic effects which are similarly enhanced by elevated temperature, upon them as upon vertebrates.

**Dual Function of Hemoglobin.** The expected increase of saturation of arterial and particularly venous blood resulting from high oxygen pressure was first observed by Bert<sup>40</sup> and confirmed in extensive studies on human subjects by Hill.<sup>95</sup> Increase of physically dissolved oxygen as well was demonstrated by Wolff and Lennox<sup>172</sup> in cats at about 1 atmosphere, and by Behnke *et al.*, whose experiments are fully discussed below.

One hypothesis explaining the toxic effect of excess oxygen in the intact mammalian organism resulting from these observations, and first proposed by Gesell,<sup>88,89</sup> postulates that part of the "dual function" of hemoglobin is lost owing to the fact that blood oxygen in physical solution is sufficient in whole or in part for metabolic needs; hence little or none is supplied by reduction of the hemoglobin. The mechanism by which this loss produces its effects is illustrated by Figure 1. This shows the relation between the total  $\text{CO}_2$ , the pH, and  $\text{PCO}_2$  of the whole blood. The arterial point, A, is on the totally oxygenated line, and the blood (the subject breathing air) contains 50 vol. % of  $\text{CO}_2$ , the pH = 7.400 and the  $\text{PCO}_2$  is 40 mm. Hg. (Assume for simplicity that the blood is 100 % saturated and contains hemoglobin equivalent to 20 vols. % of oxygen.) The venous point is derived thus: in the normal subject at rest, the mean  $\text{CO}_2$  content

of *venous* blood is 55 vols. %, approximately 5 vols. % greater than the  $\text{CO}_2$  of *arterial* blood. If the respiratory quotient is average, *i. e.*, 0.8, there will be  $5/0.8 = 6.2$  vols. of oxygen consumed per 100 cc. of blood. The blood will then be 69% saturated, and the  $\text{CO}_2$ -pH line of the whole blood will be that indicated by the dashed line intermediate between the fully oxygenated and fully reduced lines. Hence the *venous* point will be

### TOTAL $\text{CO}_2$ , WHOLE BLOOD, VOLS. %

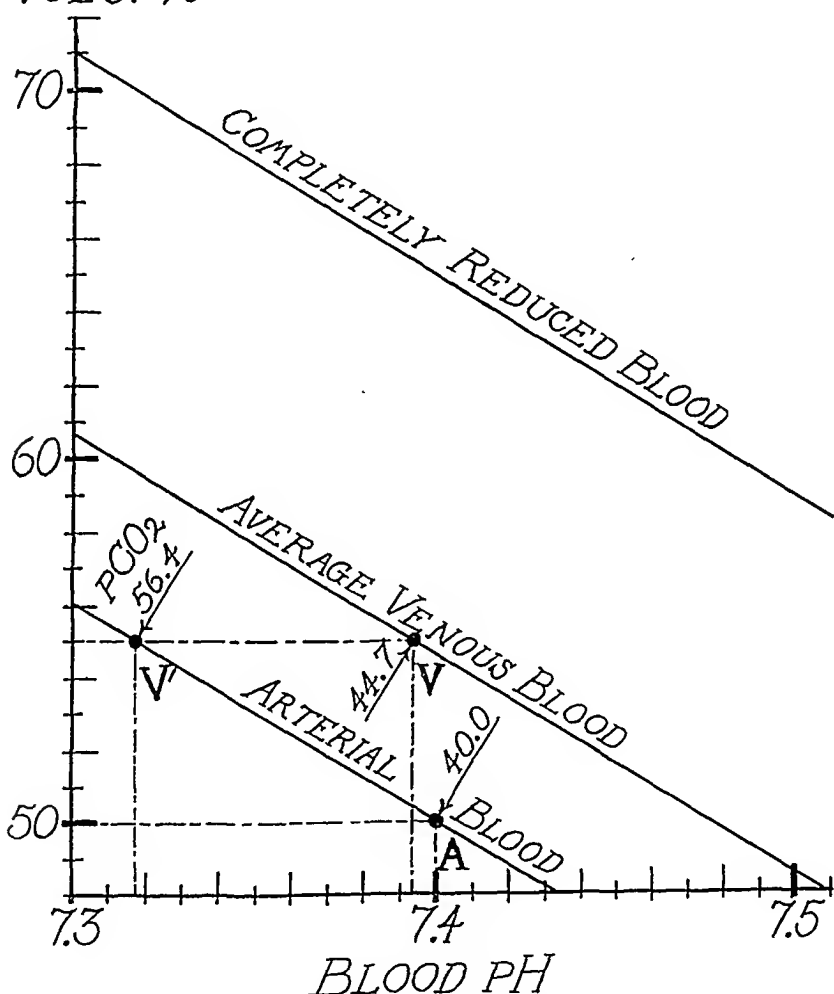


FIG. 1.—Schematic diagram illustrating the loss of the dual function of hemoglobin when sufficient oxygen (2.6 atmospheres) is inhaled to supply metabolic needs by physical solution.

at V'. The pH and  $\text{PCO}_2$  will then be 7.395 and 44.7 mm. Hg respectively. By reduction of the hemoglobin, 4.73 vols. % of  $\text{CO}_2$  (95% of the total) are taken up without any change in pH, while only 0.27 vols. % (5% of the total) are taken up by the pure buffer action of the blood. The pH change is thus very small, *i. e.*, only 0.005 pH. The situation is quite different if the subject is breathing oxygen under increased pressure. For example, the solubility of oxygen in water is 2.4 vols. % per atmosphere

and hence (neglecting the diminished solubility in blood since the calculations are approximate and illustrative only) at  $6.2/2.4 = 2.6$  atmospheres oxygen pressure the oxygen in physical solution alone would be sufficient to take care of the metabolic needs. There would be no reduction of the hemoglobin whatever and the line showing the relation between total  $\text{CO}_2$  and pH of the venous blood would be the fully oxygenated one. The venous point would then be at  $V'$  and the pH and the  $\text{PCO}_2$  would be 7.317 and 56.4 mm. Hg respectively. The elimination, owing to the presence of sufficient physically dissolved oxygen for metabolic needs, of this important function of hemoglobin, namely, release of base upon reduction, increases the changes of the pH by 0.078 and of  $\text{PCO}_2$  by 11.7 mm. Hg beyond those occurring normally. The increased metabolism of exercise does not affect the above conclusions. For it is easy to show, on physico-chemical grounds only, that when the  $\text{CO}_2$  output and oxygen uptake increase *pari passu* the calculated values of pH and  $\text{PCO}_2$  of the venous blood are essentially those given for the basal state.

If this mechanism is the sole one operating when oxygen at high pressures ( $>2.6$  atmospheres) is inhaled, we would expect to find a decrease below the normal venous pH of about 0.08, and an increased venous partial pressure of  $\text{CO}_2$  of about 12 mm. Hg. Bean,<sup>18</sup> using the manganese dioxide electrode, was successful in measuring such changes of pH of blood in dogs breathing oxygen at elevated pressures, the venous blood being arterial in color. No  $\text{PCO}_2$  values were reported. Bean, in agreement with Gesell, attributed considerable significance to these changes. Campbell has reported extensive observations on the partial pressure of  $\text{CO}_2$  in the tissues of animals breathing air, and oxygen under high pressures. He injected bubbles of air or nitrogen into tissues or into the peritoneal cavity and after sufficient time had elapsed for equilibration with tissue  $\text{CO}_2$ , removed the gas for analysis. He found quite consistently, compared to normal conditions, that under high oxygen tensions the partial pressure of the  $\text{CO}_2$  in tissue was elevated by 15 to 20%. This led him to conclude that a major effect of oxygen poisoning is a "retention" of free carbon dioxide by the tissues. Paine, Lynn and Keys,<sup>17</sup> on the other hand, studying the gases in artificially obstructed intestinal loops of dogs in 1 atmosphere of oxygen, found no changes in the carbon dioxide levels from those found in similar animals breathing air, although the oxygen tensions rose as was anticipated. These observations are suggestive, but the critical experiment to test Gesell's hypothesis requires determination by the glass electrode of the actual pH, the analysis of the arterial and venous blood for total  $\text{CO}_2$ , and the calculation from these data of the  $\text{PCO}_2$  of the venous and arterial blood under conditions of high oxygen pressure.

Behnke *et al.*<sup>36</sup> have approached this ideal experiment so closely that their observations may be accepted with confidence. Arterial and venous blood from dogs under high oxygen pressures was analyzed for total  $\text{CO}_2$ . Samples of the same blood were then equilibrated with known pressures of  $\text{CO}_2$  and the total  $\text{CO}_2$  content determined. From these data the authors constructed  $\log \text{CO}_2 - \log \text{PCO}_2$  lines according to the method of Peters,<sup>139</sup> and by interpolation determined the  $\text{PCO}_2$  of the original arterial or venous samples. The pH values could then be calculated by the use of the familiar Henderson-Hasselbach equation. Although indirect, this method of calculation rests upon a sound basis. Behnke reports the following findings in the case of 9 dogs breathing oxygen at about 3.8 atmospheres:



1. In all cases the venous oxygen content was equal to or greater than the oxygen capacity indicating that the entire metabolic oxygen was supplied by physically dissolved oxygen. That is to say, there was no reduction of the hemoglobin and the mechanism under consideration was operative.

2. The arterial  $\text{PCO}_2$  was either the same (7 observations) or slightly lower than when breathing air. This is proof that carbon dioxide *transport* is not interfered with.

3. The venous  $\text{PCO}_2$  was significantly higher than the controls breathing air by an amount averaging 6.5 mm. Hg greater than the controls.

4. The pH of the arterial blood was unchanged whereas that of the venous was less but by a small amount averaging about 0.03 unit.

Behnke concluded from these data that this unique form of  $\text{CO}_2$  acidosis expected on physico-chemical grounds does occur but that it is slight, and he dismisses this loss of the dual function of hemoglobin from consideration as a causal factor in oxygen poisoning.

The reasons given by Behnke (somewhat extended by the authors) are: (1) the familiar symptoms of  $\text{CO}_2$  acidosis do not resemble remotely those of oxygen poisoning; (2) the changes observed are slight; (3) according to the theory maximal oxygen poisoning should be reached at 2.6 atmospheres, since at this pressure the dual function is completely lost. But Behnke emphasized that oxygen poisoning frequently does not develop promptly at pressures of 3 to 4 atmospheres despite this complete loss. Only 2 out of 9 of his dogs had convulsions even when the hemoglobin of the venous blood was completely saturated. Moreover, Behnke and others have reported that at higher pressures (4 to 6 atmospheres) toxic symptoms are severer and occur sooner than at lower pressures (see section on Tolerance).

One may conclude from the evidence at hand that the changes in the blood expected on physico-chemical grounds do occur when excess oxygen is inhaled, but that they play little or no rôle in the picture of oxygen poisoning.

**Enzymatic Systems.** The conviction is growing that the action of oxygen is upon the enzyme systems of cells resulting in reversible or irreversible changes such that serious impairment of essential cellular metabolic functions results. The idea goes back at least to Paul Bert's time: Although he found that pressures of oxygen up to 15 atmospheres had no influence upon the activity of such enzymes as salivary diastase, pepsin, invertase, emulsin and myrosinase, he did show that a strip of beef muscle suspended in oxygen at similar pressures showed diminished oxygen and  $\text{CO}_2$  metabolism compared to controls under normal conditions. He also found that putrefaction was delayed or inhibited by high oxygen pressures.

The literature on the action of high oxygen upon enzymes may for convenience be discussed in two parts: (1) scattered, relatively non-systematized observations showing the effect of oxygen upon sundry enzymes; (2) more or less systematized studies upon one enzyme forming the basis of an hypothesis explaining oxygen poisoning. These latter are discussed in connection with the hypotheses proposed.

The enumeration of the scattered studies follows:

Confirming Bert's observation on enzymatic autolysis are the studies of Bailey *et al.*<sup>13</sup> and also Laquer.<sup>117</sup> McCance,<sup>129,130</sup> however, found that the effect of aerobic autolysis was mainly upon urea formation which was diminished under oxygen. Meyer<sup>131</sup> showed that the ability of mouse brain homogenates to oxidize guaiacum in the presence of hydrogen per-

oxide was greatly diminished by a preliminary exposure to 4 atmospheres of oxygen for 4 hours. Shapiro and Wertheimer<sup>150</sup> reported that fatty acid dehydrogenase demonstrated by the Thunberg technique in rat tissue is peculiarly susceptible to short exposures to oxygen. Marks,<sup>123,124</sup> and Marks and Fox<sup>126</sup> found that the catalase activity of extracts from marine animals and plants compared to anaërobic controls is significantly diminished by prolonged exposure (1 to 2 days) to air or oxygen. However, oxygen inactivation of catalase is very slow (20 days for complete inactivation at 25° C.).

One difficulty in interpreting the effects of oxygen upon enzymes is illustrated in the case of arginase and urease. Both of these enzymes in impure preparations are inactivated with more or less rapidity by exposure to oxygen, but when purified the inhibiting action is much less.<sup>80,81,93,116,146</sup> In purification, the removal of catalytic amounts of heavy metals—which may catalyze the oxidative inactivation of enzymes—must be borne in mind as a possible cause for their increased resistance.

Gale<sup>87</sup> found that formic dehydrogenase from *B. coli* was inactivated by oxygen. The inactivation was shown not to be due to the production of H<sub>2</sub>O<sub>2</sub>, oxalic acid or formaldehyde.

Lehman<sup>119</sup> studied the effect of oxygen pressures and pH upon succino-oxidase activity. In general maximum activity was found at comparatively very low oxygen tension, varying somewhat with pH but averaging 49 to 50 mm. Hg. At higher tensions the activity decreased. Bohr and Bean<sup>45</sup> found that preliminary exposure of succino-oxidase from pig heart to oxygen at 5 to 7 atmospheres decreased the subsequently measured activity (as measured by the Thunberg method) from 10 to 50%. No restoration of activity occurred after standing in air. In connection with these examples of the inactivating action of oxygen upon enzymes, attention is called to the point made by Brooks<sup>52</sup> that oxygen may be both inhibitor and reactor; in certain inorganic as well as biochemical reactions it is known to play this dual rôle.

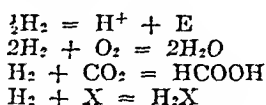
In the following discussion of hypotheses of the toxic effect of oxygen upon enzymatic action, the names given to the hypotheses are those of the Reviewers. In most cases the original work was not done as a primary study of oxygen poisoning. The Reviewers have placed their own interpretation upon the experimental data, but have been careful to distinguish in their discussion their own from the original authors' opinions.

*The "Active Oxygen" Hypothesis.* Libbrecht and Massart<sup>121</sup> reported studies on succino-dehydrogenase. They constructed a pressure chamber to contain a manometric apparatus of the Warburg type. Small motors in the pressure chamber controlled from the outside manipulated the apparatus. To prevent burning out of the motors, the chamber was filled with air or nitrogen at high pressure, but the respiratory vessels proper, being connected to small balloons containing oxygen, were filled at high pressures (5 atmospheres) upon elevation of the pressure in the chamber. In the case of a freshly prepared succino-oxidase system, the authors found that 5 atmospheres of oxygen completely stopped oxygen uptake in the presence of succinate. That the true dehydrogenase activity was blocked was shown by the fact that the further addition of methylene blue did not restore oxygen uptake. However, aged preparations, or those treated with cyanide to inactivate the cytochrome oxidase system, were unaffected by high oxygen pressure since with succinate such preparations took up oxygen actively provided methylene blue was added to replace the lost cytochrome system as oxygen acceptor. From these

observations, Libbreeht and Massart concluded that molecular oxygen *per se* is non-toxic but that the cytochrome oxidase system together with high oxygen inactivates irreversibly the succino-dehydrogenase by "l'oxygène actif." The term "l'oxygène actif" is too vague a conception for current acceptance. Perhaps the hypothesis could be re-framed by stating that the cytochrome system together with oxygen at high pressures oxidizes the succino-dehydrogenase to an inactive form. This unique hypothesis of oxygen poisoning receives no support from the experimental work of Stadie and associates.<sup>161</sup>

*Hypothesis of Reversible Oxidation of Ferrohemochromogens.* This well-substantiated and interesting hypothesis of oxygen poisoning centers around the enzyme, hydrogenase.

Hydrogenase catalyzes the exchange reactions:



It is present in a variety of bacteria and algæ, but not in mammalian tissue. Stephenson and Stickland,<sup>162</sup> who first described the enzyme, observed that it was inactivated by molecular oxygen, as did Wieland and Pistor,<sup>163</sup> Claren,<sup>71</sup> and Gaffron.<sup>86</sup> Wilson, Lee and Wilson<sup>171</sup> studying the hydrogenase activity of azotobacter revealed a miniature picture of the oxygen problem in mammals. As the oxygen tension rose from low levels the activity of the hydrogenase system increased rapidly since oxygen was playing the essential rôle of reactant. But an optimal oxygen tension was soon attained beyond which a toxic zone was reached wherein activity diminished and finally ceased.

It is upon the interesting and important experiments of Hoberman and Rittenberg<sup>102</sup> that the hypothesis under discussion is based. Using deuterium to measure the activity of the hydrogenase from *proteus vulgaris* they found that the inactivation by oxygen was completely reversible. Reversal could be brought about slowly by re-equilibration with deuterium, and rapidly by reducing agents (hydrosulfite), or biologic agents such as glucose, pyruvate, succinate, formate, or fumarate. The authors concluded that hydrogenase is an iron hemochromogen which can exist in either the reduced or oxidized state. Oxygen inactivation is due to the formation of inactive ferrihemochromogen; reversal of inactivation is brought about by reduction to the active ferrohemochromogen; rapidly by reducing agents, and slowly by deuterium. In further support of this conclusion is the fact that cyanide inactivates under aerobic but not under anaerobic conditions. This was attributed by the authors to the well-known fact, as in the case of peroxidase and catalase, that reduced iron hemochromogens do not combine with cyanide. This attractive and well-substantiated hypothesis of the mechanism of oxygen inactivation of an enzyme cannot, however, explain all toxic action of oxygen. It is even impossible to conclude that all iron or metallo-hemochromogens will react similarly, since in unpublished experiments Stadie, Riggs and Haugaard<sup>161</sup> have found that catalase, a hemochromogen enzyme, is resistant to prolonged action of oxygen at high pressures.

*Hypothesis of Action of Oxygen upon Co-enzymes: Glyoxalase.* This enzyme catalyzes the dismutation of methyl glyoxal to lactic acid and is abundantly present in mammalian tissue. Reduced glutathione functioning as a co-enzyme is necessary for its action. Jowett and Quastel<sup>110,111</sup>

showed for the glyoxylase of human red cells and rat tissues a progressive inhibition of enzymatic activity in the presence of oxygen which they attributed to the formation of the inactive oxidized form of glutathione.

This appears to be the only known instance of an enzyme system inhibited by the action of oxygen upon the co-enzyme. However, the case of cysteine and carboxypeptidases studied by Irving, Fruton and Bergmann<sup>106</sup> (see *infra* under Formation of Enzyme Inhibitor by Molecular Oxygen for discussion) is also properly discussed here. Cysteine, according to these authors, forms a complex with the enzyme which thereupon acquires activity. The oxidation of the cysteine by molecular oxygen to cystine results in inactivation.

*Hypothesis of Inactivation by Oxidation of Activating Metal in Enzyme.* This possibility is illustrated by the studies of Warburg and Christian on yeast zymohexase.<sup>167</sup> The enzyme is an important one in carbohydrate metabolism since it catalyzes the splitting of hexose diphosphate into 2 molecules of triose phosphate. The enzyme requires free reduced Fe, Cu, or Co for activation. The action of oxygen is through cysteine in the following way: in the presence of a small amount of cysteine molecular oxygen oxidizes the metal, in which state it is no longer activating. In the case of zinc, which also activates the enzyme, but exists only in one valence state, oxygen and traces of cysteine are without influence. A second mechanism of inactivation by cysteine must be mentioned although not related to oxygen action: an excess of cysteine either anaërobically or aërobically inactivates because of complex formation removing the activating metal.

However, the possibility is remote that this particular mechanism of oxygen action on important carbohydrate metabolic processes is a factor in oxygen poisoning in mammalian organisms, since the zymohexase prepared from muscle is completely independent of metals for activity and in consequence cysteine and oxygen are without influence upon its activity. However, as a prototype, similar oxygen-enzyme interaction may exist in mammalian tissue although the Reviewers have found no references to such.

*Formation of Enzyme Inhibitor by Molecular Oxygen.* An interesting form of oxygen inactivation is that discussed by Irving, Fruton and Bergmann<sup>107</sup> in the case of swine kidney and beef spleen carboxypeptidases. Experimentally they used the dipeptide carbobenzoxyglycylphenylalanine as substrate and studied its rate of hydrolysis. Cysteine is required for the activation of these enzymes. Oxygen may inactivate by oxidizing the cysteine to cystine which no longer activates (*v. supra*), similar to the case of glyoxalase. But another type of inactivation may result by the action of molecular oxygen, according to the authors. In the first case, if the enzyme was preliminarily equilibrated in the presence of oxygen with cysteine before the addition of the substrate, a diminished but constant reaction rate was obtained. In the second case, if the preliminary equilibration period was omitted, the aërobic digestion began at almost as high a rate as the anaërobic digestion, but this rate diminished to the same low but constant rate as that observed in the first case. This inhibition, the authors concluded, was not due to oxidation of cysteine, which was present in excess and remained essentially unchanged, but cysteine reacted in the course of the incubation with an unknown component of the crude swine kidney extract to form an inhibitor for the carboxypeptidase. Swine kidney pepsinase which requires no activator (*i. e.*, cysteine) was not inactivated by oxygen. These experiments not only illustrate an interesting possible mechanism of oxygen inhibition of enzymatic systems,

but also show that a sulfhydryl activator such as cysteine may react in two ways with molecular oxygen to influence enzyme action: (1) by being itself oxidized and thus losing its function as an activator; and (2) by reacting together with precursors to produce enzyme inhibitors.

*Action of Oxygen Upon the Sulfhydryl Enzymes.* Recently there has been a renewed interest in the so-called sulfhydryl enzymes. These enzymes are assumed to be active or inactive depending upon the state of the sulfur as a constituent part of the enzyme molecule. Two possibilities are recognized: (1) The sulfur exists in the reduced or sulfhydryl form, designated commonly as  $\text{EnSH}$ . In most cases (insulin is an exception) the enzyme is active in this state, hence the term, sulfhydryl enzymes. (2) The enzyme may contain the sulfur in the oxidized or dithio form, usually designated by the symbol  $\text{EnS:SEn}$ , in which condition (excepting insulin) the enzyme is inactive. The transformation of one state to another is in most cases reversible and may be brought about on the one hand by oxidizing agents such as porphyrindine, iodine, iodosobenzoate, oxidized glutathione, iodoacetamide, and so forth, or, on the other hand, by reducing agents such as cysteine, reduced glutathione, thioglycolic acid, hydro-sulfite,  $\text{HCN}$ ,  $\text{H}_2\text{S}$ , etc. Reversible inactivation may also be brought about by the use of certain mercaptan reagents, *viz.*: cuprous oxide, mercurials such as chloromercuribenzoic acid, and organic arsenicals which presumably react with the  $-\text{SH}$  group producing mereptides according to the equation:  $\text{EnSH} + \text{X} = \text{EnS}\cdot\text{X}$ .

The possibility that oxygen at ordinary or high pressures acts as the oxidizing agent to produce enzymatic inhibitions by this mechanism is obviously real, and indeed there is experimental evidence in the case of succino-oxidase that such is the case. This hypothesis, therefore, becomes important in the discussion of the mechanisms of oxygen poisoning.

For discussion of the literature on the sulfhydryl enzymes, reference is made to the review article of Hellerman<sup>92</sup> and the recent articles of Barron and Singer<sup>16</sup> and Potter and DuBois.<sup>141</sup> Only that portion which is pertinent to the subject of oxygen poisoning will be discussed here.

Hellerman<sup>92</sup> reported that urease and arginase—two sulfhydryl enzymes—are inactivated by oxygen but the inactivating action of oxygen is less on the purified enzyme presumably because catalytic copper was removed. Since succino-dehydrogenase is the best studied enzyme in relation to oxygen effects, a more extended discussion is in order. Hopkins and Morgan<sup>104</sup> showed that succino-dehydrogenase, whose action is independent of a co-enzyme, is completely inactivated by the addition of oxidized glutathione. Upon further addition of reduced glutathione or cysteine, complete reactivation is observed. Hence, they concluded that succino-dehydrogenase is a sulfhydryl enzyme and that activity is dependent upon the reduced form— $\text{EnSH}$ . In later studies, Hopkins *et al.*<sup>105</sup> made the important observation that malonic, fumaric and succinic acids protected the enzyme from the oxidizing action of the glutathione. Potter and DuBois<sup>141</sup> studied in detail the mechanism of the catalytic action of succino-dehydrogenase. They agreed with Hopkins and Morgan that the action is dependent upon the presence of the  $-\text{SH}$  groups as constituent parts of the enzyme molecule. They picture the enzyme as being in an equilibrium state between oxidized and reduced form, *viz.*:  $\text{EnSH} \rightleftharpoons \text{EnS} + \text{H}$ .

Alternate oxidation by the cytochrome oxidase system and reduction by substrate explains the mechanism for the oxidation of succinic acid. They further suppose that the active  $-\text{SH}$  groups are situated between two "affinity" points which combine with the two carbonyl groups of

the succinic acid to hold it for the duration of the action of the oxidative process. Malonic acid also combines with these two points and "covers" the active center —SH group, thus protecting it from the oxidative action of oxidized glutathione or other oxidizing agents. The inactivating action of high pressure oxygen upon succinodehydrogenase has already been discussed. That the mechanism of inactivation is similar to that of glutathione is indicated by the fact that the oxygen inactivation is reversible in part by reduced glutathione, but more significantly by the fact that malonate protects completely.<sup>161</sup>

Whether the inactivating action of high oxygen by this mechanism is a universal manifestation with sulfhydryl enzymes remains to be determined by further experimentation.

*Oxidation-reduction Potential of Environment on Enzyme Activity.* That enzyme activity may be influenced by non-specific effects such as change of the oxidation-reduction potential of the medium is indicated in the recent work of Sizer and Tytell,<sup>156</sup> and Sizer.<sup>155</sup> They altered the oxidation-reduction potential of their media by the use of a variety of poisoning agents over a wide range and found that the activity of crystalline urease and yeast invertase was a continuous function of the potential. Urease showed a maximum activity at +0.15 volt; invertase showed a constant activity from -0.27 to +0.60 volt, above which it fell off sharply.

Whether or not the oxidation-reduction potential of mammalian tissue can be altered by high pressures of oxygen is unknown; but the possibility that such a mechanism may be operative in oxygen poisoning is mentioned for the sake of completeness.

Summarizing the data on enzymatic systems, the likelihood is increasing that the toxic action of high pressures of oxygen will be explained in the light of inhibitory actions on enzymes with resultant severe disturbances of essential metabolic cellular reaction. The evidence in the literature is scanty and non-systematized, but is sufficient to suggest the following possible modes of action of oxygen: (1) Oxidation of a co-enzyme to the inactive oxidized form. (2) Oxidizing activating sulfhydryl compound. (3) Oxidizing active —SH groups of enzyme molecule proper. (4) Oxidation of metallo-hemochromogen to inactive oxidized form. (5) Oxidizing activating metal constituent. (6) Formation of inhibitor from precursor other than the enzyme. (7) Inhibition of enzymatic activity by changing oxidation-reduction potentials of medium.

**Summary.** The literature on the subject of oxygen poisoning is reviewed under the following headings: (1) Symptomatology, (2) Pathologic Anatomy, (3) Blood, (4) Circulation and Peripheral Vessels, (5) Metabolism, (6) Blood Gas Equilibrium, (7) Influencing Factors, (8) Tolerance, Adaptation and Oxygen Therapy, (9) Oxygen Aero-embolism from Rapid Decompression, (10) Isolated Surviving Tissue, (11) Neoplasia, (12) Microorganisms, (13) Dual Function of Hemoglobin, and (14) Enzymatic Systems.

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## NEUROLOGY AND PSYCHIATRY.

UNDER THE CHARGE OF

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## RECENT CONTRIBUTIONS TO WAR NEUROLOGY

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RECENT literature in the field of war neurology has been largely devoted to the following topics: (1) head injuries, including those resulting from blast; (2) peripheral nerve injuries, including the subject of causalgia and related states; (3) meningococcic meningitis, which has recently risen to epidemic proportions in both military and civilian life; and (4) the use of the electroencephalogram as related to war.

While subjects of spinal cord injury, heat stroke and tropical diseases involving the nervous system may properly be considered here, the paucity of recent contributions has not justified their inclusion. Neither are subjects of neurologic interest in the sphere of aviation medicine included in this review.

**Head Injuries.** Traumatic disorders of the head may be divided into two general groups, (a) closed injuries and (b) open injuries. According to Symonds<sup>32</sup> closed head injuries include all trauma without direct penetration of the dura. In this type of injury symptoms are usually those of generalized cerebral disturbance with signs of disordered mental function. Surgical intervention is seldom required except with delayed onset of neurologic signs and increase in mental disability. Such manifestations are presumptive evidence of epidural or acute subdural hemorrhage and exploration through 4 burr holes in the skull is indicated, providing the patient's general condition permits. Of the cases of closed head injury admitted to the hospital, 60% returned to duty. The incidence of post-traumatic epilepsy is small when compared with penetrating wounds. Denny-Brown<sup>11</sup> has also discussed the principles of treatment of closed head injuries. Concussion, contusion, and hemorrhage may occur either

\* Now on active service.

independently or in combination. As a result, clinical manifestations are varied and difficulties in therapy are encountered. Indications for treatment are discussed as follows: (1) Rapid increase in coma or in signs following initial improvement is indicative of an extradural hemorrhage, and immediate surgical intervention is indicated. When retrogression is slow, acute subdural hemorrhage or subdural hygroma is a likely possibility. According to Denny-Brown, the results of surgery in these latter conditions are poor. Conservative management is usually indicated, as the subdural hemorrhage of hygroma is frequently of secondary importance to concussion of the brain. Increase in psychomotor activity is rarely observed when a compressing mass is of significant size. (2) An increase in spinal fluid pressure accompanied by deepening coma is an indication for exploration. However, a moderate increase in pressure without an increase in the depth of coma is of no especial significance. Denny-Brown believes that increase in pressure is most frequently due to impaired venous drainage, not cerebral edema. (3) In the group of patients in which symptoms and signs decrease, increased pressure and localizing signs are absent and the electroencephalogram contains diffusely abnormally slow activity, surgical intervention is not indicated.

In cases of cerebral concussion, supportive measures, rest and the use of oxygen will usually lead to recovery.<sup>10</sup> In the unconscious patient the importance of keeping the respiratory passages open cannot be minimized. He should be placed on his side or even on his abdomen with the head turned toward one side, thus insuring easy breathing and the escape of saliva. Mock and Mock<sup>24</sup> advocate the transportation of these patients in the prone position; suction apparatus should be available. Dependent drainage is of help in some patients, according to Peet.<sup>24</sup> Utilizing animal experimentation, Faulkner<sup>14</sup> points out that in cases of head injury with bleeding from the mouth and nose, respiratory difficulties may be the result of blood entering the trachea and lungs rather than due to involvement of the respiratory center as is commonly assumed. Morphine is avoided by most observers because of its depressing effect on the respiratory center and its tendency to increase intracranial pressure.<sup>24,27</sup> Horrax,<sup>21</sup> however, states that small doses may be given preoperatively if the patient is not unconscious or extremely drowsy. Mock and Mock<sup>24</sup> believe that all types of disturbing influences should be avoided during the first 6 hours, which is the most critical period. These include suturing of wounds, oversedation, the use of antitetanus serum or even transferring the patient to a better room. The patient should be placed between blankets, heat applied and oxygen given as indicated.

The use of dehydration and spinal drainage in cases of head injury associated with increased spinal fluid pressure is still a controversial subject. Denny-Brown,<sup>11</sup> for instance, maintains that dehydration is generally of no benefit and may be of harm. Mock and Mock have surveyed the management of 6544 consecutive cases of head injury throughout this country and feel that the moderate use of dehydration and spinal drainage have proved themselves to be of value. Symonds<sup>32</sup> advocates the use of lumbar puncture as a guide to therapy. If the pressure is 250 mm. or more, withdrawal of fluid is indicated. When headaches associated with increased pressure are not alleviated by raising the head, hypertonic solutions by mouth or rectum may be employed. Rowbotham<sup>31</sup> feels that the determination of the spinal fluid pressure and the presence of subarachnoid bleeding by means of lumbar puncture is important in the management of the acute brain injury. Lumbar puncture should be per-

formed after 12 hours and fluid removed until the pressure reaches 50 mm. of water. If the initial pressure is high, the pressure should be repeated as often as every 4 hours. Rowbotham advocates the moderate use of 50 % sucrose solution and magnesium sulfate enemas as dehydrating agents. Spinal drainage without dehydration is used when subarachnoid bleeding has occurred. Rogers<sup>29</sup> prefers the use of magnesium sulfate rectally rather than intravenous methods. Magnesium sulfate should not be employed intravenously. Turner<sup>31</sup> reports success with the use of concentrated serum as a dehydrating agent.

Cairns and Holbourn<sup>7</sup> describe the advantages of the use of crash helmets by motorcyclists. Head injuries have occurred in 41 % of a series of motorcycle accidents. The crash helmet, made of either vulcanized rubber or preferably compressed wood pulp, tends to spread the blow over a wide area and prevent lacerations, fractures and brain contusions. It also spreads the blow over a longer period of time, thus diminishing the rate of deceleration of the brain.

Horrax<sup>21</sup> divides head injuries with penetration of the dura into: (1) gutter wounds, in which the missile has made a furrow through the scalp and caused fragments of bone, hair and clothing to penetrate into the brain; (2) simple penetrating wounds, which also carry débris into the brain; (3) penetrating wounds from multiple, usually small, fragments which are especially common in this war; (4) perforating injuries, with débris scattered along the track; and (5) injuries involving air sinuses. In the first 2 types contaminated scalp and contused brain are removed. A soft rubber catheter is inserted into the track in order to locate fragments. The area is cleaned by suction. Experience in this war has shown that multiple, small penetrating fragments are best left alone. Perforating or through-and-through injuries are usually fatal. In survivors, there is usually little to do. Limited débridement may be indicated, and accessible bone fragments should be removed. Injuries involving air sinuses are especially serious because of the continued source for infection. Closure of the dura is imperative when possible. If this is not possible, muscle implants or gauze packs may be used.

Experience has shown that innocent-looking penetrating scalp wounds are potentially dangerous and require careful attention. Botterell and Jefferson<sup>6</sup> state that one-quarter of the air raid casualties admitted to an English hospital had scalp wounds. Blood was found in the spinal fluid in one-fifth of the cases of wounds apparently confined to the scalp. Emergency treatment, according to Craig,<sup>10</sup> should consist of shaving the scalp, cleansing the wound with a minimum of manipulation, introduction of sulfanilamide powder and application of a sterile dressing. Extensive operative procedures should be delayed until adequate facilities are available, a neurologic examination has been made and Roentgen rays of the skull taken. With the use of sulfonamides, wound closure may be delayed from 24 to 48 hours.

Miller,<sup>23</sup> citing his experiences in this war, strongly condemns the failure to perform primary suture of head wounds. The open treatment is frequently followed by intradural infection and should be used only in cases of long standing with obvious infection. In some cases uncomplicated healing is obtained with wounds older than 48 hours. He advocates the transfer of patients with head injury to a center where competent care is available. Bone or metal fragments that cannot be found and sucked out without the use of Roentgen rays should be left alone. Because of this he states that Roentgen rays are not always essential prior to the operation.

Cloward,<sup>9</sup> treating the first American casualties of this war in Hawaii, notes that nearly all head injuries were associated with compound depressed fractures of the skull. These were caused by sharp, irregular shell fragments apparently traveling at high rates of speed; as a result many of them penetrated into the brain substance. Cloward points out that most of the patients with penetrating wounds did not lose consciousness. This tends to confirm the experimental studies of Denny-Brown and Russell<sup>13</sup> on concussion. They have shown that the movement of the brain must be accelerated at least 28 feet per second in order to produce unconsciousness. Cloward expresses the belief that small fragments enter the skull at such a high rate of speed that generalized acceleration of the brain is not produced. As Kennedy<sup>22</sup> has also noted, when consciousness is lost as a result of this type of trauma it is by reason of damage to extensive areas of brain and not merely the result of concussion. The prognosis in unconscious patients with penetrating head injuries is therefore poor. Among the patients so injured in Hawaii, all of those admitted to the hospital died within a few minutes to 12 hours. Cloward mentions a few important points upon which most authors agree in the treatment of penetrating wounds of the head: "1. The optimum time to operate is within 6 hours after the injury, but wounds which have gone untreated as long as 48 hours should be treated as fresh wounds and closed without drains, unless there is an obvious infection. After 48 hours the wound is treated as a brain abscess with open drainage. 2. All metallic foreign bodies should be removed as soon as possible, because they lead to secondary abscess and epilepsy. 3. The defect in the dura mater should be closed completely in clean wounds with fascia or periosteal grafts. 4. Generous use of sulfanilamide not only in the scalp and skull wound but in the missile tract in the brain is recommended. Sulfanilamide is preferred because it has been found to be much more rapidly absorbed into the blood stream from an open wound than any of the other sulfonamide drugs."

Munro<sup>26</sup> emphasizes that operation should not be performed unless the general condition is satisfactory. Treatment for shock is begun immediately and débridement is delayed until such treatment is effective. Munro condemns shaving and washing the wound until the patient is ready for operation. Diagnosis is made by palpation through the wound. The wound is then covered with a sterile gauze dressing and further manipulation is avoided until the patient is ready for operation. Complete débridement should be performed within 48 hours after the injury or else should be delayed for 6 to 8 months. Suction rather than irrigation is the most effective method of wound cleansing; irrigation tends to spread infection. Drainage is not necessary in any wound in which débridement has been properly performed. While Munro recommends the use of sulfonamides, he believes that they should be used only as an adjunct to proper surgical treatment. Bone or metal fragments lodged in the brain should be removed if accessible. However, if removal necessitates further damage to normal brain, non-interference is the best policy. Electromagnets have been utilized in the removal of metal.<sup>9</sup> Money and Nelson<sup>25</sup> feel that it is more important to remove bone fragments and inorganic débris than metallic foreign bodies. The dura mater should be closed in order to prevent the development of herniation of the brain, cerebrospinal fluid fistula or aërocele. Dural defects may be repaired by use of a periosteum patch obtained from the overlying skull.<sup>9</sup>

The mortality rate of 60 % in cases of head injury during the early stages of the first World War, which was later cut in half by Cushing's improve-

ments in surgical technique, is evidently becoming even smaller in the present war. This is largely made possible by the local and systemic employment of the sulfonamides. Carmody,<sup>8</sup> for instance, reports that all of 12 patients with major gunshot wounds of the head recovered in spite of the fact that 10 of them had severe infections on admission to the hospital. This recovery is attributed in large measure to the local application of sulfanilamide powder supplemented with parenteral sulfadiazine. Watt and Alexander,<sup>35</sup> however, report the frequent occurrence of generalized or focal epileptic seizures following the local application of sulfathiazole to the brain. The local use of this particular drug is therefore contraindicated. No such complications have been encountered with sulfanilamide, sulfapyridine or sulfadiazine.

A variety of substances have been utilized in the repair of bony defects in the skull. Bone grafts have been preferred, but various types of metal plates, celluloid and cartilage have been used. Objections have been raised to all of them. The discovery by Venable and Stuck that vitallium, an alloy of cobalt, chromium and molybdenum, produces no tissue reaction and has tremendous tensile strength has been utilized by Geib<sup>17</sup> and Beck.<sup>5</sup> Geib reports the use of vitallium in the repair of skull defects using casts of metal previously designed to fit the defect. Beck has modified the procedure by employing stock plates that can be used without preliminary casting. More recently Pudenz<sup>28</sup> and Fulchar<sup>16</sup> have reported the use of tantalum. This metal is said to be non-irritating, easily malleable, inelastic, usable as extremely thin plates, and non-absorbable.

*Blast Injuries.* The effects of blast on the organism are profound. These are mainly in the lungs and, particularly in the case of immersion blast, the gastro-intestinal tract. There are, however, relatively few reports on the effects of blast on the central nervous system. Most observers have felt that such effects are not conspicuous and are of secondary importance to those in the chest and abdominal cavity.<sup>15</sup> Ascroft<sup>4</sup> describes numerous petechial hemorrhages in the cerebral cortex of a person dying from the nearby explosion of a hand grenade. Zuckermann finds that intracranial hemorrhages are confined to the pia and the tela choroidea in experimental animals subjected to blast.

Anderson<sup>3</sup> has discussed the psychiatric disturbances found in 8 persons exposed to the detonation of high explosive bombs. He expressed the belief that impairment was due to structural changes in the brain, but that constitutional or psychogenic factors may have coexisted and deserve consideration in the plan of therapy. In general, periods of unconsciousness following the blast were absent or mild. Impairment of memory was the most characteristic feature. Lack of concentration, forgetfulness, lability of affect, apathy, transient aphasia, depression and dementia were noted.

Abbott, Due and Nosik<sup>1</sup> have observed 10 cases of subdural hematoma or subdural effusion of spinal fluid following blast injury. The patients present a history of exposure to severe concussion, loss of consciousness for a period varying from a few minutes to several days, persistent headaches, memory loss and irritability. Neurologic signs are not conspicuous. "The most pronounced symptoms are persistent headaches, usually generalized (increased on exertion and often nocturnal), a history of coma, syncope or convulsions which did not exist prior to the blast, and a definite departure from a stable personality." When the lesion is suspected, pneumoencephalography is indicated. "If a hematoma or effusion is present in the subdural space, a characteristic filling defect is observed over the

cerebrum with an occasional distortion of the ventricular system." The total protein of the spinal fluid may be increased. Removal of the entrapped fluid led to prompt recovery. Five of the cases were bilateral.

In another report<sup>2</sup> the same observers have described the psychiatric picture in greater detail and the use of psychologic testing in diagnosis. Two syndromes are distinguished. The first and more common is one in which pronounced retardation in intellectual activity and interpersonal relationships occurs. The person tends to be dull and lacks "push." The second syndrome is seen in the more chronic cases; its most striking feature is impairment of inhibitions, with euphoria, motor restlessness and emotional lability. The Rorschach test and especially the Shipley-Hartford Retreat test for intellectual impairment<sup>30</sup> have been useful adjuncts in arriving at the diagnosis. In the latter procedure a vocabulary test, not easily affected by structural disease, is contrasted with an abstraction test, which is readily impaired by such changes. The authors conclude that "intellectual impairment in blast cases with or without positive neurologic findings is an indication for a pneumoencephalogram."

Blast injuries following underwater explosion present a particular problem. Experiments by Greaves *et al.*<sup>19</sup> illustrate the danger of having the head below the surface during the blast. Whereas guinea pigs swimming on the surface had no macroscopic brain lesions, 4 guinea pigs with heads submerged, all had extensive epidural and subdural hemorrhages. One had a skull fracture; 2 had hemorrhages extending into the spinal canal. Hamlin<sup>20</sup> describes the clinical syndrome in immersion blast injuries as consisting of clouding of the sensorium and diminished reflex activity commensurate with the severity of the visceral and pulmonary injuries. In some cases red cells or an increase in the total protein may appear in the spinal fluid. The mechanism of the production of neurologic symptoms is still in the theoretical stage. Hamlin suggests that the blast wave may be transmitted to the neuraxis so that cerebral acceleration is produced as in cerebral concussion. It is probable, however, that the neurologic difficulties are due more frequently to transient ischemia from the rapid shift in vascular reservoirs. He notes that all survivors within the danger zone of an underwater detonation will have had their heads above the water when the compression occurs. Pain in the limbs, testicles, abdomen and chest is thought to be the resultant of forces acting on the peripheral and autonomic nerves.

*Chronic Effects of Brain Injury.* Denny-Brown<sup>12</sup> has reviewed his experience with 400 cases of prolonged disability following head injury mainly as the result of the war. Psychoneurotic symptoms were found to be more frequent problems than "organic" defects; 262 cases were suffering from psychoneurosis; 22 from traumatic epilepsy, and 116 from other organic defects. Overemphasis is too frequently placed upon either the structural or the emotional factors. Satisfactory therapeutic results require attention to both factors. Denny-Brown expresses the belief that psychoneurotic symptoms may result from structural damage following severe injury. These symptoms may also be a consequence of attempting to adjust to a pre-traumatic level in the presence of intellectual impairment. When no evidence of severe injury is obtained, one can usually elicit a history of previous emotional instability. In cases of concussion, the duration of amnesia following the trauma is the most reliable indicator of cerebral damage.

Important factors in prognosis, according to Symonds,<sup>32</sup> are: (1) the pre-traumatic constitution, as gauged by the family and personal history;

(2) age; (3) duration and degree of mental disorder following the injury; (4) duration of traumatic amnesia; and (5) the particular situation of the individual, including his desire for return to duty, anxiety regarding future capacity and compensation factors. Reassurance, occupational therapy and graduated increases in activity are useful therapeutic agents.

Symonds and Russell<sup>33</sup> state that the prognosis in 718 cases of chronic head injury admitted to a military hospital because of unsatisfactory progress was twice as poor as in their acute cases. They believe that a greater predisposition to mental disorders existed in this group. The prognosis in 111 members of the Royal Air Force with acute and chronic head injuries was 4 times as good as in the entire series. These men were highly selected as far as mental and emotional disorders were concerned.

The recent publication of Kurt Goldstein,<sup>18</sup> "Aftereffects of Brain Injuries in War," emphasizes the important problems that will be encountered in the care of the more or less permanently disabled cases of head injury. The book is divided into two parts. The first deals with the general, neurologic and mental symptoms and with a variety of testing procedures used to elucidate more obscure defects. The second part is concerned largely with the treatment of aphasic and agnosic disorders and the principles employed in determining subsequent placement and the patients.

Epilepsy, Goldstein notes, is a frequent sequelæ of penetrating skull wounds; grand mal and petit mal attacks occur in 15 to 20 % of all patients and other varieties are common. Attacks occurring shortly after the injury are less inclined to become chronic than those appearing after 1 or 2 years. Many of the early seizures are associated with irritative factors that can be eliminated, while late attacks are frequently associated with scarring. He points out the importance of careful perimetric examination in these patients. Visual defects may be due not only to focal lesions of the visual apparatus, but also to diffuse blows; or they may be an expression of abnormal fatigue. Central vision usually improves earlier than peripheral, so that the resultant tubular constriction may be confused with that seen in hysteria. Disturbances in space perception and in color vision are also noted.

Frontal lobe involvement is common. Extreme akinesia with apparent intactness of the memory and other mental functions may be noted; sometimes it is associated with catalepsy. Abnormal postures and disturbances in coördination are observed. Aphasia, apraxia and related phenomenon may be due to lesions in the second and third frontal convolutions. Goldstein describes the mental changes in frontal lobe lesions as an impairment in the capacity of "abstraction;" the patients become more "concrete in their behavior." "The abstract attitude is basic for the following potentialities: 1. Assuming a mental set voluntarily. 2. Shifting voluntarily from one aspect of a situation to another, making a choice. 3. Keeping in mind simultaneously various aspects of a situation. 4. Grasping the essential of a given whole, breaking up a given whole into parts and isolating them voluntarily. 5. Abstracting common properties, planning ahead ideationally, assuming an attitude toward the 'merely possible,' and thinking or performing symbolically. 6. Detaching the ego from the outer world. In all these potentialities, the patients are more or less impaired."

Another group of symptoms arise not directly from the damage to the brain but from "the struggle of the changed organism to cope with the defect and to meet the demands of a milieu with which it is no longer able

to cope." Behavior as a whole therefore differs fundamentally according to the situation. In the face of a situation to which he cannot adapt, the patient may exhibit a variety of mental, emotional and vasomotor phenomena. Goldstein calls this a "catastrophic situation." Numerous psychologic laboratory examinations have been carried out in order to evaluate the mental functions, the general level of performance, the presence of circumscribed mental defects such as aphasia, and to study the working capacity in special kinds of labor. The mere results of tests, such as the intelligence quotient, are ambiguous. The procedure in which the patients arrive at them, however, is of more significance. In the tachistoscopic examination a variety of letters, words, figures, numbers, objects and colors are projected upon a screen for a fraction of a second. One-tenth second, for instance, is ordinarily required to recognize familiar objects. In cases of brain damage the time required to recognize them may be lengthened. "With this plan one can find slowing down of mental processes, disturbances of attention, of memory, of the visual capacity in different directions, disturbances of reading (alexia), of visual recognition of objects (agnosia), as well as defects of the visual field, disturbances of color vision, aphasic symptoms." Ergographic tests are used for the study of fatigue and working capacity. The observation of labor in hospital workshops is of use not only in retraining but also in giving indications of the patient's future work capacity.

The section on therapy is devoted to a detailed discussion of the re-educational training procedures employed in a variety of aphasic and related disturbances. Training is based on the previous careful evaluation of the patient by clinical and laboratory methods. Treatment, in general, is devoted to retraining functional mechanisms, as in childhood, or, in case the brain apparatus is so damaged that former performances are irrevocably lost, by building up substitute mechanisms.

In patients discharged after treatment, Goldstein notes that while but 20% became almost normal workers, only 18% did nothing and earned no money.

**Peripheral Nerve Injuries.** Injuries to peripheral nerves are an important problem in war medicine. Among 383 injuries treated in the surgical service of an American naval hospital, comprising casualties from the Solomon Islands engagement, there were 30 peripheral nerve injuries.<sup>40</sup> In this group the nerve injuries were frequently associated with compound fractures or with wounds that were not healed sufficiently to allow an anastomosis. Therapy, therefore, was devoted to promoting healing and avoiding contractures rather than nerve suture.

Seddon<sup>52</sup> has suggested a new classification of peripheral nerve injuries: (1) Complete anatomic division (neurotmesis). Prompt surgical intervention is indicated in this group. (2) Complete loss of function without anatomic disturbance of the internal structure (axontmesis). Differentiation between this and the first type ordinarily can be made only by exploration. When no anatomic division is seen, regeneration occurs more rapidly and completely; there should be no interference with the nerve. (3) Transient block (neurapraxia). This group is characterized by partial loss of function which is mainly motor in character, little wasting of musculature, absence of a reaction of degeneration, the presence of paresthesias and relatively rapid recovery of function without surgery.

The character and treatment of peripheral nerve injuries encountered in war has been discussed by Craig.<sup>39</sup> He notes that war injuries present a more difficult therapeutic situation than those ordinarily seen, inasmuch



as tissue destruction is usually greater and suturing is delayed longer. In spite of the new methods of treatment that are being developed at the present time, Craig expresses the belief that severed nerves are best treated by end-to-end anastomosis. If possible, this should be done without tension, hemorrhage, or infection. He advocates the use of local and systemic sulfonamides. "The sulfonamides do not inhibit nerve regeneration but allow for the suture of infected or potentially infected nerves, thus shortening the period which formerly was allowed for the infection to clear." Suturing is ordinarily performed with silk or cotton. Steel and tantalum, which produce no tissue reaction, are proving to be of value. "Nerve grafts are as yet not as efficacious as end-to-end suture, which may be achieved by multiple operations with lengthening of the nerve by wide dissection and primary suture of the separated nerve ends with flexion of the neighboring joints." Craig states that massage and passive motion are useful in keeping the muscles in good condition, but they do not stimulate nerve regeneration. The radial nerve appears to be most frequently involved. Wounds of the median nerve are most often complicated by the development of causalgia. Injury to the brachial plexus is commonly the result of concussion, hemorrhage or stretching rather than laceration. It is therefore best to delay operative procedures with this type of wound in the hope that spontaneous recovery will ensue.

According to Learmonth and Wallace,<sup>46</sup> peripheral nerve injuries are frequently associated with scarring due to tissue destruction and sepsis. They believe that active coöperation between the neurosurgeon and the plastic surgeon is essential to proper therapy. After neurologic examination, the plastic surgeon should obtain a covering of normal skin and subcutaneous tissue for the damaged area. Exploration of the nerve is carried out later.

The rate of nerve regeneration following operation has been calculated by Marble, Hamlin and Watkins.<sup>48</sup> The average rate in cases of ulnar nerve injury was 1.24 mm. daily, with the results varying from 3.6 to 0.58 mm. daily. The average rate in radial nerve injury was 1.09 mm. daily, with a range of from 2 to 0.5 mm. They point out that in nerve injuries associated solely with fractures, nerve suture is seldom indicated.

A variety of new techniques in nerve grafting have been reported in the last few years. Nerve grafting was formerly restricted for the most part to the use of fresh grafts from non-essential nerves of the injured patient. The use of coagulable plasma as a glue to replace ordinary sutures was reported by Young and Medawar in 1940.<sup>55</sup> The plasma was obtained from cockerels and chick embryo was added as the clotting agent. This "fibrinogen-rich glue" was placed in the gap between the nerves and formed a bridge across which new axons would grow. Bentley and Hill<sup>57</sup> described the experimental use of transplants from other animals of the species. Tarloff and Benjamin<sup>53</sup> have modified the method of Young and Medawar by using autologous plasma fortified with autologous muscle extract. They believe that this preparation results in less subsequent inflammatory reaction and scar formation.

Weiss and Taylor,<sup>54</sup> in attempting to overcome the difficulties of using preserved nerves as grafts, have devised a method in which the nerves are frozen and dehydrated and then stored aseptically. They found that when the nerves were rehydrated that they assumed their normal histologic appearance and could be successfully grafted into animals of the same

species. They suggest that "banks of assorted nerve sizes stored in the dried condition could readily fill a steady demand."

de Rezende<sup>41</sup> reported the use of peripheral nerves obtained from cadavers in bridging nerve gaps in experimental animals. The first clinical application of this procedure was made by Klemme, Woolsey and de Rezende.<sup>44</sup> The grafts were suspended in a solution of formaldehyde, transferred to alcohol 2 or 3 days before the operation, and immersed in a saline solution  $\frac{1}{2}$  hour before the operation. The graft was fixed in place using a 50% acacia glue and an allantoid membrane was placed around it.

Neuroma formation following peripheral nerve injury is commonly attributed to the misdirected and excessive outgrowth of the proximal end of the neurones which are attempting to find a pathway down the distal portion of the nerve. Klemme, Woolsey and de Rezende point out that neuromas will form at both of the cut ends of a nerve although the proximal end is usually larger. The suggestion is made that neuroma formation is due mainly to hemorrhage with scar formation and that the growth of axons distally merely increases the size of the proximal neuroma. They emphasize that operation should be performed immediately after the injury or else after the wound has healed completely. Grafts should be used if there is any tension on the nerve; a graft may be applied later if immediate suture is unsuccessful. They point out that suturing results in laceration of many of the axis bundles and for this reason prefer the use of nerve grafts.

Richter and Katz<sup>49</sup> have described an objective method of mapping out peripheral nerve or sympathetic involvement by measurement of the electrical skin resistance. They have found that skin resistance is higher in denervated areas and that these areas are sharply demarcated with this procedure. It is possible to map out areas affected by peripheral nerve injuries without the active coöperation of the patient and is therefore an advantage in the examination of patients who are unable to coöperate and in cases of malingering. It should be of help in following the progress of nerve regeneration after suture. Richter and Katz state that the apparatus is simple and the method rapid.

Massage, motion and the application of moist heat are ordinarily advocated beginning about 2 weeks after the operation. There is no common agreement as yet on the effect of electrical stimulation in retarding the rate of muscular atrophy following denervation. Guttmann and Guttmann<sup>42</sup> and Hines, Thompson and Lazere<sup>43</sup> have expressed the belief that beneficial effects are obtained. Lavrentjev<sup>45</sup> has stated that heat is the sole means of increasing the rate of nerve regeneration. Billig and Van Harreveld<sup>38</sup> have suggested a new method for the re-innervation of chronically paralyzed muscles such as found in poliomyelitis or peripheral nerve injuries. The method is based on the fact that regeneration of nerve fibers is accompanied by branching. They therefore interrupt the remaining intact nerve fibers of a motor nerve innervating a paretic muscle and depend on the branching during regeneration to obtain an increase in the number of active muscle fibers. The interruption is accomplished by the pressure of forceps without interruption of the nerve sheath and can only be applied to motor nerves which contain few sensory fibers.

*Causalgia and Phantom Limb Pain.* Pain following peripheral nerve injury or amputation may be expected to be frequently seen in wartime. Causalgia and related states were described in detail by Weir Mitchell in soldiers injured in the Civil War. Little has been added in the intervening years in elucidating fundamental mechanisms involved. Livingston's

publication, "Pain Mechanisms: A Physiologic Interpretation of Causalgia and Its Related States,"<sup>47</sup> reviews the subject and makes provisional attempts to correlate it with recent neurophysiologic and anatomic concepts. The term causalgia ("burning pain") has been used to designate a syndrome which follows peripheral nerve injuries and is characterized by: (1) burning pain; (2) trophic changes, especially glossy skin; and (3) a local rise in temperature. Livingston believes this definition to be too restricted; all 3 are symptoms which follow peripheral nerve irritation and which may occur either isolated or in combination. Moreover, hyperesthesia and sweating are often noted, and coldness is more frequently present than heat. Persistence of the symptoms may result in thinning of the skin, fibrosis and atrophy of muscles, and atrophy of the bones with osteoporosis. He restricts the term causalgia to severe cases characterized by burning pain, employs Homan's term, "minor causalgia," for less severe cases and "post-traumatic pain syndromes" to include cases in which burning pain is not a feature. Occasionally the pain and other symptoms are reflected to the same position in the contralateral extremity as a "mirror image." This mirror image may persist after the original lesion has disappeared. Livingston states, "It is difficult to escape the conviction that some dynamic process has been initiated within the spinal cord that may persist after the original stimulus is withdrawn."

Riecher<sup>48</sup> gives the incidence of causalgia as 2% in a series of 500 cases of peripheral nerve injury. While it usually follows trauma to the sciatic or median nerve, injury to other nerves may give rise to the phenomenon. It occasionally appears after wounds in which no opening of the skin or fracture has occurred. The pain is aggravated by contact with anything that is dry. A central disturbance is indicated by the fact that emotional experiences may precipitate an attack. Gask and Ross's view that pain from peripheral nerve irritation is due to the excessive liberation in the skin of an "H-substance" which increases skin sensitivity is not necessarily disproved by the relief of pain by sympathectomy. Vasodilation following this procedure may in effect assist in washing away the substance.

The term "phantom limb" is applied to the sensation of the persistent presence of a limb after it has been amputated.<sup>47</sup> This sensation may, indeed, be more vivid than when the extremity was intact. Sometimes, severe pain is felt in the phantom limb, most frequently of a burning character. Occasionally, the patient feels as if he is able to voluntarily move the fingers or toes, or they are felt to be in a cramped position. In the presence of phantom limb pain, the stump is commonly cold. Hyperesthesias and sweating may be present. Fibrillary twitches and clonic contractions are observed in some stumps, and they may be increased with emotion.

Bailey and Moersch,<sup>46</sup> in a study of 105 patients, note that phantom limb appears after most amputations (90.5%) but becomes incapacitating in only a few. Among 56 amputations necessitated by trauma, the sensation occurred immediately in 42 and was delayed in the remainder. Symptoms did not become distressing, however, until later. This period averaged 5 years, though as long as 29 years elapsed. Of 15 varieties of surgical and non-surgical therapies none gave anything but transient relief. The authors could find no single theory to explain the phenomenon. They concluded that it probably is of psychogenic origin, possibly a type of "obsession neurosis."

Riddoch<sup>50</sup> believes that impulses from the stump are fundamentally responsible for the sensation. The impulses attempt to maintain an image

of the intact "body scheme" in consciousness. These excitation impulses are subject to inhibitory processes in the central nervous system. If the peripheral excitation is intense and the central inhibitory processes are weakened, painful sensations may result. Thus, ill-health or emotional disturbances may precipitate the painful state by reducing central inhibition.

Livingston, likewise, believes that the fundamental etiologic factor in all of these conditions lies in irritation arising from the traumatized peripheral nerve. In proposing a theory to explain the phenomenon one must take into account the fact that eliminating the focus of irritation does not always abolish the pain. Livingston utilizes the concept of self-re-exciting internuncial pools of neurons in which nerve impulses may continue to discharge after the stimulus has ceased; this type of neuronal arrangement has been suggested by neurophysiologists as an explanation for after-discharge. He summarizes, "An organic lesion at the periphery, involving sensory nerve filaments, may become a source of chronic irritation. Afferent impulses from this 'trigger point' eventually create an abnormal state of activity in the internuncial neuron centers of the spinal cord gray matter. The internuncial disturbance in turn is reflected in an abnormal motor response from both the lateral and anterior horn neurons of one or more segments of the cord. The muscle spasm, vasomotor changes, and other effects which this central perturbation of function brings about in the peripheral tissues, may furnish new sources for pain and new reflexes. A vicious circle of activity is created. If the trigger point is removed early, the process may subside spontaneously. If the process is permitted to continue, it spreads to involve new areas, and tends to acquire a momentum that is increasingly difficult to displace. Perhaps in this stage, even a removal of the original irritant may not be sufficient to establish a cure. But if an important part of the circle of reflexes can be interrupted, the process may subside, and a normal physiologic status is again established. If again the pathologic patterns gain the ascendancy, the repeated breaking of the circle may result in a permanent cure." Therapy should therefore not be delayed. Frequently novocaine injection or surgical removal of the trigger point helps to break up the vicious circle. If this does not succeed, interruption of the sympathetic pathways, either by injection of sympathetic ganglia, periarterial sympathectomy or sympathetic ganglionectomy, may be of benefit. If in turn this approach is unsuccessful, posterior rhizotomy or anterolateral chordotomy may be attempted.

Livingston does not subscribe to the view, held by Foerster and Leriche, that sympathectomy relieves pain by interruption of sensory fibers, inasmuch as experimental evidence indicates that all such fibers pass through the posterior roots. Lewis submits that vasodilation and increased blood flow is the only explanation available at the present time for benefits from sympathectomies. While not denying the importance of the vasomotor factor, Livingston is not completely satisfied with this explanation, feeling that it is but a part of a more fundamental central disturbance.

**Meningococcic Meningitis.** In a review of the health of the U. S. Army, Simmons<sup>64</sup> states that the army has experienced an outbreak of meningitis which is similar to, though milder than, that in the first World War. A sharp peak of 4.5 cases per 1000 per annum was reached in 1918. A similar abrupt rise to 2.7 has occurred in this war and has been maintained for a longer period than in the first World War. The incidence in the civilian population has increased fivefold over the previous 5 year average.

The army outbreak began at approximately the same time throughout the country and was of more serious consequence in camps with a large turnover. The case fatality rate has decreased from 34% in World War I to the present rate of between 3 and 5%. This is chiefly due to the use of sulfonamides.

Taranto<sup>66</sup> has made an analysis of the symptomatology found in 100 consecutive cases of cerebrospinal fever at a navy camp. The most prominent features were headache (90%), nuchal rigidity (80%), vomiting (64%), petechiae (64%) and prostration, delirium or coma (38%). He was impressed by the paucity of neurologic findings; the Babinski sign, for instance, was elicited in but 5% of the cases. Only 1 patient succumbed. Sulfadiazine was of inestimable value. In most cases 4 gm. were given by mouth on admission, followed by 2 gm. in 3 hours, 2 gm. in another 3 hours and then 1 gm. every 4 hours until the temperature was normal for 72 hours. Delirious patients were given sodium sulfadiazine intravenously. The average period of therapy was 8 days. Incoming raw recruits were most susceptible to the disease. With the arrival of a new battalion, the incidence increased to a peak in 3 weeks and then gradually declined. Awe, Babione and De Lamater<sup>66</sup> have reported 2 deaths in 50 cases of meningococcal meningitis seen at the U. S. Naval Hospital in San Diego. One occurred 20 minutes and the other 4 hours after admission. Eleven of the patients were comatose and 27 stuporous or irritational. In spite of the severity of the process in most cases, striking improvement was apparent within 24 hours after administration of sulfapyridine. They have emphasized that successful therapy depends upon early diagnosis and prompt use of large amounts of sulfonamides. Meningitis should be considered in every man complaining of cold and headache or backache; lumbar puncture should be performed without delay in any suspicious case. Large initial doses of sulfonamides were considered essential. "The primary dose here has been either 4 or 6 gm. of sodium sulfapyridine intravenously, followed by 2 gm. intravenously every 4 hours. This routine is continued for 3 to 5 days." This was followed by oral administration in 29 patients. Fifteen patients received serum in addition to sulfapyridine. No difference was noted between this group and the remainder who received sulfapyridine alone, except for the high incidence of serum sickness. Newcomer and Frame<sup>62</sup> have also reported 2 deaths in 50 cases and no deaths among those with 12 or more hours of treatment. Parenteral sodium sulfapyridine was used in this group.

Daniels, Solomon and Jaquette<sup>67</sup> have studied 112 patients with meningococcal infection at Fort Bragg, N. C. The incidence of infection increased markedly beginning December 30, 1942, rising to 8 per 1000 per annum for the entire camp and 20 per 1000 annually for unseasoned troops. Of all these cases, 59% were from the group of soldiers with less than 3 months' service comprising one-third of the total camp population. The authors have stressed the importance of viewing the disease primarily as a meningococcal septicemia, as it is only in this way that the diagnosis can be made before meningitis develops. Infection arises in the nasopharynx and invades the blood stream, with localization in the skin, joints, meninges and other body tissues. In 32 cases a diagnosis of meningococemia without meningitis was made. While upper respiratory infection, headache and nausea were among frequent symptoms of meningococemia the most characteristic manifestation was the rash. Most commonly it was petechial or purpuric. Ill-defined pink macules and maculopapular lesions were also seen. The initial blood culture was positive for men-

ingococci in 56 %; therefore in many cases diagnosis had to rest on clinical grounds. Prompt therapy prevented the advent of meningitis.

In the 80 cases of meningitis, bacteriologic confirmation was obtained in 97.5 % of the patients who had not received any sulfonamide previously. On the other hand, no meningococcus was grown in any patient previously given the drug. Only 1 death occurred in the entire group. Sodium sulfadiazine, intravenously, was the drug of choice in cases of meningitis. One group received 0.1 gm. per kg. intravenously as an initial dose and one-half this amount every 8 hours thereafter until it could be retained by mouth. Another group received an initial dose of 0.05 gm. per kg., 0.025 gm. per kg. 4 hours later and then the latter amount every 8 hours. The smaller dosage was equally effective, and the incidence of renal complication from sulfadiazine decreased.

Hill and Lever<sup>60</sup> reported on 68 cases of meningococcic infection treated at Camp Edwards, Mass. Intravenous sulfonamides, especially sulfadiazine, were used. Not a single death resulted. The meningococcus was frequently recovered from clear spinal fluids.

Thomas<sup>67</sup> reviewed a series of 1518 cases treated in the Fourth Service Command. The early mortality rate of 8.8 % in 317 cases was later reduced to 2.1 %. Two-thirds of the cases and 80 % of the fatal cases were noted in new, unseasoned troops.

The American experiences correspond quite well with those reported in British troops. For instance, Priest<sup>68</sup> reported the same "miraculous" results with the sulfonamide treatment of the 204 cases which occurred among the British Expeditionary Forces in France. Only 2 patients died, in neither case directly due to the effects of the meningococcus. In general, the cases were of 3 types: (1) fulminating onset with loss of consciousness and late appearance of meningeal signs; (2) acute onset with immediate signs of meningitis, the most common type; and (3) onset with chronic meningococcic bacteremia.

**PROPHYLAXIS.** Thomas<sup>67</sup> has stated that the carrier rate is between 1 and 2 % in non-epidemic periods and rises to 30 % or more during epidemics. Gray and Gear<sup>59</sup> have expressed the belief that if the carrier rate exceeds 20 % in a military camp, the mass use of sulfapyridine is indicated. Fairbrother<sup>58</sup> had previously demonstrated its effectiveness in the elimination of carriers. Sulfadiazine and sulfathiazole have proved themselves equally effective as prophylactic agents. Sulfanilamide apparently has not been as useful.<sup>65</sup> Kuhns *et al.*<sup>61</sup> administered sulfadiazine prophylactically to more than 15,000 soldiers. Following the administration of 3 gm. daily for 3 days or 2 gm. daily for 2 days, the incidence of cerebrospinal fever in the group fell abruptly. Only 2 cases occurred during an 8 week period of observation, while 40 cases occurred among 18,800 untreated controls. One of the 2 treated patients was admitted to the station hospital for measles and developed meningitis 9 days later; the other was transferred to an untreated organization after he had received the drug.

**Use of Electroencephalography in the Armed Forces.** Schwab<sup>71</sup> states that the usefulness of the electroencephalographic examination of navy personnel falls into 3 main categories: (1) in examination of applicants for naval service, the examination being confined to those men with histories of fainting attacks or head injury; (2) in examination of men actually in the service who have fainted or have had seizures; and (3) in determination of the extent of intracranial damage to members of the personnel who have suffered head injuries. Williams<sup>72</sup> studying an English

military group found 40% abnormalities among men with head injuries, 26% abnormalities in psychoneurotics and 5% abnormalities in a group of military aviators. Ten per cent of the records in a control group were classified as abnormal. Harty, Gibbs and Gibbs<sup>68</sup> have reported on the electroencephalographic examination of 275 candidates for military service. Thirty per cent of the records were abnormal, contrasted with 15% in a control group. However, when 127 men in the group with a history of severe head injury or neuropsychiatric disorder were eliminated, the percentage dropped to 13. The greatest percentage of abnormalities were found in psychopathic personalities (88%) and in head injuries with neuropsychiatric disorders (85%). In 6 candidates with a family history of epilepsy, 4 were abnormal, while in 4 with seizures in infancy 2 were abnormal. Of cases classified as "mixed neuropsychiatric disorders," 61% were abnormal. Among men with head injuries, 26% with a history of unconsciousness following the injury had abnormal records, while but 11% were abnormal in those in which consciousness was unimpaired. The authors concluded that electroencephalographic examination is advantageous in cases in which the results of other tests are equivocal or in which a general examination indicates that a man is borderline between acceptable and rejectable.

In chronic post-traumatic syndromes, Heppenstall and Hill<sup>69</sup> relate the abnormalities in electroencephalographic findings to the duration of post-traumatic amnesia, the time elapsed between the injury and the recording, and to abnormal family and personal histories. Sixty-three per cent of the records were abnormal in cases of predominantly "organic" syndromes and 37% in predominantly "functional" syndromes. Denny-Brown<sup>12</sup> states that improvement in the electroencephalogram in cases of concussion corresponds roughly with the clinical condition of the patient. The record may not be of value, however, if it is unrelated to clinical data.

McDaniel *et al.*<sup>70</sup> report on the use of the electroencephalogram at the U. S. Naval Hospital in Philadelphia. They state that it is proving itself at least as useful to the neuropsychiatrist as the electrocardiogram is to the cardiologist. Solomon *et al.*<sup>72</sup> also believe electroencephalography to be a valuable instrument in the selection of recruits, especially those with the diagnosis of epilepsy. Among 432 patients, they state that the electroencephalogram was of primary importance in arriving at a decision in 10%, of secondary importance in 53% and of incidental value in 37%.

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## PHYSIOLOGY

### PROCEEDINGS OF

### THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF NOVEMBER 16, 1943

**Determination of Serum Total Base by Ion Adsorption.** B. D. POLIS and J. G. REINHOLD (Division of Biochemistry, Philadelphia General Hospital). A simple, rapid method for total base determination was



described based on the adsorption of the cations by Amberlite Resin 1R100 and the titration of the acid effluent with standard base.  $\text{CO}_2$ , which is partially lost, is removed by aëration with  $\text{CO}_2$  free air. The base bound as bicarbonate is calculated by a  $\text{CO}_2$  determination. This added to the value determined by titration gives the total serum base. The method showed high precision and good accuracy compared with the electro-dialysis method of Keyes, except in the extreme acidic range of 0-10 mcq.  $\text{HCO}_3$ . In this range the results, though low, are consistent enough to apply a correction factor.

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**The Blood Picture of Athletes as Affected by Intercollegiate Sports.** EDMOND J. FARRIS (The Wistar Institute of Anatomy and Biology).<sup>\*</sup> Over 300 blood counts were made on college athletes before and after match games. In most of the 8 sports an emotional lymphocytosis was evidenced before the game, with concomitant reduction in the neutrophils.

In football, basketball and baseball the relative lymphocytosis disappeared during the course of the game. In wrestling and track the lymphocytosis increased during the course of the event. In tennis, golf and rowing, the relative lymphocytosis remained about the same throughout the event. Some indication of the physical condition of each player was furnished by the differential blood count picture after football, basketball and baseball games. Blood counts of players fatigued to exhaustion ranged from 80 to 91 % neutrophils.

Intensity of activity in competition, together with duration, produced a leukocytosis in all events. A certain intensity in activity was essential to produce the marked leukocytosis, in spite of duration.

The total number of erythrocytes per c.mm. was increased in the short term athletic events, and usually decreased in events which lasted over 25 minutes.

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**Clues Regarding the Organization of the Simplest Living Systems.** STUART MUDD (Department of Bacteriology, University of Pennsylvania). The microbiologic parasites may be arranged in a series of diminishing size and metabolic independence. Bacteria are able to synthesize certain growth accessories, lipids, carbohydrates, nucleic acids and proteins. It must be admitted that no pattern of organization of sufficient refinement and complexity has as yet been detected as a possible structural basis for these exacting chemical syntheses. Nevertheless, electron microscopy has shown a considerable degree of organization of the minute bacterial cell. A cell wall in the solid state is separable from an inner fluid or potentially fluid protoplasm. Extracellular capsules, flagella, spores and spore-like bodies, intracellular granules and areas of differing densities are all, when present, capable of demonstration under favorable conditions.

Pathogenic rickettsiæ and viruses have either lost or never achieved their metabolic independence. They are obligatory intracellular parasites. The studies of Plotz, Smadel, Anderson and Chambers with rickettsiæ and of Green, Anderson and Smadel with vaccinia (which is one of the largest viruses), nevertheless demonstrate simple cellular organization. Cell walls and differentiation within the protoplasm are clearly seen in these rickettsiæ and in the vaccinia elementary bodies. Structural differentia-

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<sup>\*</sup> This investigation was aided by a grant from the Samuel S. Fels Fund.

tion, indeed, has been recorded by Luria, Delbrück and Anderson in micrographs of even smaller parasites, namely in the heads of coli bacteriophage particles, which are only about 65 to 80  $m\mu$  in diameter.

Plant viruses would seem to be at the bottom of the microbiologic scale of parasitism. The viruses of tobacco and cucumber mosaic disease have been prepared by W. M. Stanley and others as crystalline macromolecules of nucleoprotein. Analysis by Roentgen ray diffraction by Bernal and Fankuchen has shown the molecules of tobacco mosaic virus to have a definite internal architecture due to regular periodic arrangement of the structural units of which the macromolecule is composed. Tobacco mosaic virus possesses the property of reproduction in suitable hosts and of "mutation," including change in chemical constitution; these are two attributes which have hitherto been considered distinctively characteristic of living systems. It may well be questioned, however, whether a crystalline molecule can be considered to be living.

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**An Apparatus for Study of the Physiology of Air-borne Infection.** W. F. WELLS (Laboratories for Study of Air-borne Infection, University of Pennsylvania). In experiments on air-borne tuberculosis the number of tubercles in the lung corresponded with the estimated number of bacilli-bearing nuclei breathed by rabbits, and their general distribution indicated ready penetration to the remotest passages. The physical behavior of air-suspended particles, which determines their penetration to the lung, is governed by the relation between surface area and weight. Friction (proportional to surface area) holds particles in air currents, while mass deposits them on surfaces. Their state of suspension can therefore be defined by settling velocity (measured by the number of particles which settle on unit area in unit time divided by number per unit volume).

The solid content of a droplet determines the size of its particulate residue. Settling velocity of nuclei can be regulated by the solid content of droplets of constant size produced by a special atomizer. The atomizer nozzle inserted tangentially at the equator of a horizontal flask whirls larger droplets to the flask wall where they return to the pool. The air is rapidly saturated by the evaporation of a constant number of the smallest droplets, and their nuclei are swept out with the air stream from the core of the flask air. The constancy of both size and number depends upon the saturation of a constant supply of air of constant humidity by droplets of constant size. Loss in flask weight determines the amount of atomized fluid, gauge readings the amount of compressed air used, and bacterial sampling the number of droplets. Settling velocity (or particle size) is estimated from simultaneous Petri plate and centrifuge samples.

The number of particles in the air breathed by the animals is maintained constant by chimney action—draft being created by a special gas burner supplied by the infected air. Any system connected to this chimney will be under negative pressure to the combustion chamber, reassuring safety of persons in the room. Air flow is estimated from readings of wet and dry bulb thermometers in the air line.

A hexagonal infection chamber is connected by a 2-inch hose line to the chimney and to the atomizer flask. Each of the 6 sides is fitted with a port, to which a plethysmograph can be attached, so that the head of the

animal projects into the chamber. Influent air rises around the edges of a false floor, passes the heads of the 6 animals facing each other, and discharges through a central outlet, effluent air being sampled by an air centrifuge exhausting the outlet line. A small ultraviolet light sterilizes the air at the termination of exposure and reduces superficial contamination of the animals and interior surfaces. A kymograph standing on top of the chamber records air displacement. From the measurement of air breathed by the animals, and the bacterial content of the air, the number of inspired particles can be estimated, and by comparison with the number of colonies in the lung, the penetration of defensive barriers can be correlated with particle size.

# BOOK REVIEWS AND NOTICES

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**BAROMETRIC PRESSURE.** Researches in Experimental Physiology. By PAUL BERT. Translated from the French by MARY ALICE HITCHCOCK, M. A., Formerly Professor of Romance Languages at the University of Akron; and FRED A. HITCHCOCK, PH.D., Associate Professor of Physiology at The Ohio State University. Pp. 1055; 23 tables; 89 figs. Columbus, Ohio: College Book Company, 1943. Price, \$12.00.

It is the fate of most individual contributions to knowledge to be washed by the years into a heap of anonymity—many of them forever, and some to be long forgotten. The rescue of great classics depends not alone upon their greatness, but also upon that small, select group of alert minds who, not content to let the heap lie still, search out the Vesalii, the Harveys, and the Paul Bert's for our illumination and inspiration. Paul Bert's narrow escape from these misty reaches of dim, forgotten time is due less to the global war which has brought the physiology of altered atmospheric pressure into prominence than to the timely perspicacity of the translators, to whom humanity must be grateful for the rebirth of a classic of orderly, scientific investigation during a time of utter chaos. In the preface of the original edition (1878), Bert does not exaggerate in saying, "The present book, if I am not mistaken" (and indeed he is not) "holds interest not only for physiologists, but also for physicians, engineers, and even travellers."

To touch now briefly upon the plan and structure of this *magnum opus* given us by the Hitchcocks, and to sample here and there its brightest passages will be, it is hoped, the *antipasto* which demands enjoyment of the whole feast.

The work is divided into 3 parts: history, experiments, and conclusions. Of the first part, some 500 pages, Dr. John F. Fulton says in his foreword to the translation, "We should be lastingly in (Paul Bert's) debt for this masterly presentation—a model, be it said, for any student wishing to write in the field of medical history." With this comment there can be no disagreement by the most casual reader of the book. One is led first upon a tour of the Lofty Regions of the Earth, from the Alps through the mountains of the Caucasus, Marco Polo's Roof of the World, the Himalayas, the peaks of the Americas and Africa, and the highest islands, with something of their history and measurements of their altitudes. With this orientation, the author gathers the observations of travellers on mountain journeys, quoting from the followers of Cortez in 1530, von Humboldt in 1802, and a host of others, from wandering Jesuit priests to Bert's own contemporaries. From these, and similar accounts of balloon aeronauts, he passes to a discussion of all the explanations and experiments devised to explain the symptoms suffered by those who ventured to high altitudes—symptoms which received an interesting variety of names, depending upon the age and the location. Among the earliest investigations were those of Boyle in 1670, which were followed by a dark age of mysticism and polemics lasting until the author's time, and capable of furnishing an admirable half-semester course on the questionable art of covering ignorance with polysyllabics. Of this period, it might be said again, "of the making of books there is no end, and much learning is a great weariness of the flesh." Even the illustrious von Humboldt, we are told, originated the picturesque theory that much of the disability of mountain sickness arose from the simple lowering of atmospheric pressure, which normally kept the head of the femur in its articulation, with the result that this joint was painfully weakened. This concept held much sway until Bert caused a dissection to be made, and with careful measurements, applied the simple laws of physics. By similar methods in his critical summary, the author pulverizes the unsound to extinction. The remainder of the historical section is concerned with pressures above 1 atmosphere; it contains a fascinating history of the art of

deep-sea diving in bells and suits, a topic which forms an excellent prelude to the first part of Hill's "Caisson Disease," published in 1912. In addition, one learns that the compressed air chamber was first used therapeutically in France in 1838—the *anlage* of the modern oxygen chamber. Again, the numerous theories and experiments devised to explain physiologic changes under compression are criticized and summarized, and the subject of decompression illness is given its first clear presentation.

It is frankly impossible to review or even comment justly on the second 500 pages, entitled Experiments, within a reasonable space. Of these there are some 600, presented with complete protocols (models for the modern laboratory), summaries and discussions. All the questions implied by the preceding history are undertaken for study, and the subjects used are legion: 10 species of birds, 6 of mammals, including 11 breeds of dogs in addition to mongrels, 4 species of cold-blooded vertebrates, snails and earthworms, 14 of insects, 5 of plants, numerous microorganisms, and *in vitro* preparations of tissues and enzymes. Here one may find, clearly set forth, the basis of much of our present knowledge of the oxygen-carrying function of blood under various pressures. In summarizing his experiments on rapid decompression, Bert gives the key to decompression illness and the modern practice of deep-sea diving. "Sudden decompression, beginning with several atmospheres, brings on symptoms of varying severity depending upon the degree of compression, the animal species, the individuals, and the state of the experimental animal at the time. These symptoms must be attributed to the escape of nitrogen which had been stored up in excess in the organism, following Dalton's Law." As prophylaxis he prescribes "controlled decompression," and as the only cure "a recompression, either immediate or following the inhalation of oxygen in case heart gurgles are observed." But perhaps most important of all is his discovery of the phenomenon of *poisoning by oxygen*, when, in his own words, "The oxygen which has reached a certain tension constitutes a dangerous element, often even fatal, for animal life." It is interesting that this phenomenon was little explored for half a century after its discovery.

But to proceed further with this delightful task of sampling would be unfair. Suffice it to add, again in the words of Dr. Fulton, "The third and final part . . . contains the résumé and conclusions, and is again a model of concise, orderly and logical scientific presentation."

Lest it be noted that in discussing a translation the Reviewer has devoted much space to enthusiasm over the original, it should be remembered that there would be no cause for review in the absence of a translation of an exceedingly rare volume. And the Reviewer hastens to add (having read both French and English editions), that they have skilfully reconciled the 2 most difficult tasks of translation: to render faithfully and still use the best idiom.

The service performed by Paul Bert speaks for itself; that performed by his translators cannot be too highly praised: as the "Introduction to Experimental Medicine" of Bert's predecessor, Claude Bernard, is a "must" upon the shelf of every aspirant to the rocky paths of medical research, now there is available a companion, equal "must," in the Hitchcocks' faithful rendition of "Barometric Pressure."

B. R.

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**HYPERTENSION—A Manual for Patients With High Blood Pressure.** By IRVINE H. PAGE, A.B., M.D., Director, Lilly Clinic, Indianapolis City Hospital. Pp. 80; 7 figs. Springfield, Ill.: Charles C Thomas, 1943. Price, \$1.50.

This book is written expressly for the layman and proposes to give the hypertensive "patient an insight into his illness that he may be spared some of the dismay and alarm and avoid the quackery that will await him from every side." Perusal of the book will repay the clinician as well as benefit his patient. The writer explains the purpose of the various clinical procedures used in examining the hypertensive patient, and further explains in clear and

understandable terms, what hypertension is. Finally, he tells what can be done about it. The approach is simple, direct, yet surprisingly comprehensive. In a field filled with contradictions, the author has maintained a firm footing on the underlying pathology and physiology of the cardiovascular-renal complex. This book should be gratefully received by the worried and harassed hypertensive patient, as it guides him to a satisfactory way of living and to a happier outlook upon his daily existence. D. C.

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**SELF-TEACHING TESTS IN ARITHMETIC FOR NURSES.** By RUTH W. JESSEE, Science Instructor at Bridgeport Hospital, Bridgeport, Conn. Pp. 111. St. Louis: C. V. Mosby Company, 1943. Price, \$1.50.

THIS manual, intended for elementary nursing students, fits well into the accelerated nursing school program of today. Logically graduated and stimulating, it provides for the student's self-orientation and self-study. While a larger size of type and additional laboratory exercises might further enhance its practicability, this Reviewer finds it an exceptionally valuable tool for both student and instructor. H. F.

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**CYTOLOGY AND CELL PHYSIOLOGY.** Edited by GEOFFREY BOURNE. Pp. 296; several figs. and plates. New York: Oxford University Press, 1942. Price, \$6.00.

It gives one a definite confidence in mankind and secures one's faith in Science to realize that this book was produced by British scientists during the course of the 1940-1941 air blitzkrieg on Britain. In the Editor's words, "... there are probably no chapters of which part was not written within the sound of bursting bombs." In spite of the adverse conditions under which the work was done, an excellent book has resulted. The contributors are: J. R. Baker, H. Blaschko, G. Bourne, J. F. Danielli, E. S. Horning, W. Jacobsen, R. J. Ludford, J. H. Schulman, and J. D. White. Realizing that purely morphologic investigations of cells are giving way to interpretation of structure in terms of chemical composition and function, the writers have striven to produce a critical analysis of cytologic technique and the results of physico-chemical investigation of cells. The cell surface and membranes, mitochondria, the Golgi apparatus, the nucleus and the enzyme systems are capably discussed and the final chapter, by Ludford, presents the pathologic aspects of cytology. At the end of each chapter is a bibliography and the book is fully illustrated with drawings and photomicrographs. The chief value of this volume is the compilation and correlation of work from many sources and the result is the most lucid and comprehensive presentation available of our modern concepts of the cell. D. C.

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**APPLIED ANATOMY OF THE HEAD AND NECK.** For Students and Practitioners of Dentistry. By HARRY H. SHAPIRO, D.M.D., Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University. Pp. 189; 173 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, \$5.50.

THIS is the first book of its kind, to our knowledge, since Cryer's *Internal Anatomy of the Face*, published in 1916. It is "specifically designed to relate the anatomy of the head and neck to the various fields of dentistry" for students and practitioners of dentistry. The book contains 9 chapters beginning with the examination of the patient including surface anatomy of the face, oral cavity and neck. Next is considered the development and growth of oral and associated structures including some of the more common congenital and acquired malformations, such as harelip and cleft palate, of particular interest to the dentist. After a short chapter on the skull, which contains some

excellent roentgenograms, there is one of the best sections of the book on the muscles and their relation to facial expression, orthodontic applications, movements of the mandible and their rôle in mandibular fractures. A chapter on the temporomandibular articulation contains a good discussion of the anatomic variations of the components of the joint—ankylosis and subluxation. The neuroanatomic considerations deal with the nervous structures relating to the head and neck and correlate them with pain, anesthesia and motor nerve disturbances. The blood-vessels, lymphatics and salivary glands are well covered. The final chapter discusses the structures affected in maxillo-facial war injuries and the rôle of the dentist in their care correlated with that of the plastic and general surgeon.

The many photographs, illustrations and roentgenograms are excellent and the format of the text is well arranged. L. C.

**ESSENTIALS OF PROCTOLOGY.** By HARRY E. BACON, B.S., M.D., F.A.C.S., F.A.P.S., Professor and Head of the Department of Proctology, Temple University Medical School and Hospital; Head of Department of Proctology, St. Mary's Hospital; Consulting Proctologist, Rush Hospital, National Stomach Hospital, Douglass Hospital, Mercy Hospital, and Paul Kimball Hospital, Lakewood, N. J.; Fellow, International College of Surgeons. Introduction by CURTICE ROSSER, B.A., M.D., F.A.C.S., F.A.P.S., Professor of Proctology, Baylor University, Dallas, Texas. Pp. 345; 168 illus. Philadelphia, London, Montreal: J. B. Lippincott Company, 1943. Price, \$3.50.

ALL the essentials of proctology are given in this admirably readable form. Information is sufficiently detailed to make it of value to the surgeon, yet concise enough to be of value to the student and general practitioner.

Embryologic, anatomic and physiologic considerations related to proctology are presented in a single chapter. An entire chapter is devoted to examination and diagnosis. Instruments and instrumentation are discussed.

Thorough discussion of diagnosis and treatment are given on the following subjects: fissure in ano, cryptitis, anorectal abscesses, fistula, tuberculosis, proctitis and sigmoiditis, pruritus ani, hemorrhoids, foreign bodies, injuries, tumors, lymphogranuloma venereum, inflammatory stricture of the rectum, venereal disease, prolapse and procidentia, congenital anomalies of sacrococcygeal region, papillitis, megacolon, and malformations. There are chapters on anesthesia and analgesia, and proctologic nursing.

Diagnostic and therapeutic concepts are presented in well-labeled illustrations. There are several excellent tables emphasizing points in the differential diagnosis of various anorectal and colonic diseases. L. T.

**AN INTRODUCTION TO MEDICAL MYCOLOGY.** By GEORGE M. LEWIS, M.D., Member of the American Dermatological Association, Inc.; Fellow of the American College of Physicians, of the American Medical Association and of the New York Academy of Medicine; Member of the New York Dermatological Society and of the Manhattan Dermatological Society; Associate Attending Physician (Dermatology), The New York Hospital; Assistant Professor of Clinical Medicine (Dermatology), Cornell University Medical School; Attending Dermatologist to St. Clare's Hospital; Visiting Dermatologist to Welfare Hospital, etc.; and MARY E. HOPPER, M.S., Research Fellow in Medicine, Cornell University Medical School. Second ed. Pp. 342; 77 plates (1 in color). Chicago, Ill.: The Year Book Publishers, Inc., 1943. Price, \$6.50.

THE fact that this edition became necessary within the short period of 4 years after the first speaks for the popularity of this book. This is a new edition, not a revision. New materials have been introduced, commensurate

with fresh developments since the first edition, and it is clear that many shortcomings that are inevitable in any first edition have been corrected.

The authors have done well in keeping the contents confined to mycology in its laboratory aspects, and are to be complimented in keeping it up to date. It continues to be unique and adequate in its field and indispensable to the dermatologists.

F. W.

**KAISER WAKES THE DOCTORS.** By PAUL DE KRUIF. Pp. 158. New York: Harcourt, Brace & Co., 1943. Price, \$2.00.

HENRY J. KAISER evolved a plan of prepaid medical service for the workers in his industries on the West Coast. This plan, carried out by Dr. Sidney Garfield, for the sum of 7 cents a day voluntarily contributed by Kaiser's men, gives them complete medical attention. According to the author, who writes with almost exhausting enthusiasm, the plan is very successful and should "wake the doctors" (i. e., those not already awakened) to the possibilities of medical care being equitably distributed among rich and poor alike in all sections of the country. This is a timely and controversial subject which makes interesting reading.

J. L.

**RECONSTRUCTIVE SURGERY OF THE EYELIDS.** By WENDELL L. HUGHES, M.D., F.A.C.S., Hempstead, N. Y. Pp. 160; 36 plates. St. Louis: C. V. Mosby Company, 1943. Price, \$4.00.

THE various procedures used in the plastic repair of eyelids are here illustrated by cases from the author's practice. The work was originally presented as a thesis for the American Ophthalmological Society. It gives a compressive review of the literature on the subject for reference and provides a good reference work for the surgeon who has many of these cases to do.

F. A.

### NEW BOOKS

*Laboratory and Clinical Studies from the Memorial Hospital for the Treatment of Cancer and Allied Diseases.* New York City. Vol. XXIII, Parts 1 and 2. 1942. Price, 25¢ each.

*Principles and Practice of Rehabilitation.* By JOHN EISELE DAVIS, M.A., Sc.D., Veterans Administration Facility, Perry Point, Md. Pp. 211; 6 tables. New York: A. S. Barnes & Co., Inc., 1943. Price, \$3.00.

*Experimental Surgery. A Laboratory Guide for Undergraduate Students.* By J. M. McCAUGHAN, B.S., M.D., Ph.D., Assistant Professor of Surgery, St. Louis University School of Medicine, St. Louis, Mo. Pp. 82; 32 figs. St. Louis: The C. V. Mosby Company, 1943. Price, \$2.00.

The author has rendered an important contribution by preparing a detailed laboratory guide for the instruction of students in surgical principles and technical procedures. It is recognized that instruction of animal surgery sometimes appears to place undue emphasis on the importance of surgical technique without recognizing the indispensability of experience in diagnosis and surgical judgment, but the author's method of approach guards against this pitfall. The plan of instruction emphasized the fundamental considerations of inflammation and repair and opens the eyes of the students to the alterations in physiologic mechanisms which follow anatomic alterations. Each exercise concludes with a number of questions and extensive bibliography which tend to encourage thought as well as action. Since this manual is quite unique it should find wide usefulness in surgical teaching.

J. L.

*Experimental Biochemistry.* By GEORGE D. ESSINGER, M.S., Ph.D., Associate Professor of Chemistry, Chicago College of Dental Surgery, Dental School of Loyola University, Chicago, Ill. Pp. 108; 9 figs. St. Louis: The C. V. Mosby Company, 1943. Price, \$1.50.

This abbreviated laboratory manual of Biochemistry is designed for a short course. Most of the usual qualitative tests connected with carbohydrates, fats and proteins are given. No quantitative experiments for blood are included and only a few for urine. The quantitative determination of calcium in enamel is described. The manual apparently is intended for use with students of dentistry and certainly would not be suitable for a course in biochemistry given to medical students.

J. J.



*Prelude to Sanity.* By S. GREINER. Pp. 164. Published and Distributed by Master Publications (Box 647), Ft. Lauderdale, Fla., 1943. Price, \$3.00.

A highly provocative attack on current biologic views, that contains more words and theories than supportive evidence. It, however, should contain interest for those who like this type of presentation. E. K.

*A Medical Bibliography.* A Check-list of Texts Illustrating the History of the Medical Sciences. Originally compiled by the late FIELDING H. GARRISON, M.D., and now revised, with additions and annotations, by LESLIE T. MORRISON, Librarian, St. Thomas' Hospital Medical School. Pp. 412. London: Grafton & Co., 1943. Price, £2.10s. net.

*White Blood Cell Differential Tables.* By THEODORE R. WAUGH, B.A., M.D., C.M., Pathologist-in-Chief, Royal Victoria Hospital; Associate Professor of Pathology, McGill University; Consulting Pathologist, Montreal Homeopathic Hospital, Montreal, Quebec. Pp. 126. New York, London: D. Appleton-Century Company, Inc., 1943. Price, \$1.60.

This booklet of tables is successfully designed for the convenience of those having to report many "leukoocytes and differentials" and laudably desiring to give the absolute rather than the relative (percentile) numbers of the different varieties. The 100 tables range from 10 to 10,000 leukocytes per c.mm., each giving the value for each percentage. Thus, on the page for 7000 white blood cells, 1% of a given variety obviously equals 70; and 37%, less obviously, 2590. Familiarity with the book is so easily acquired that where much of this work is done, it should quickly pay time and energy dividends. E. K.

*The Human Eye in Anatomical Transparencies.* Explanatory Text by PETER C. KRONFELD, M.D., Director of Education, the Illinois Eye and Ear Infirmary, Associate Professor of Ophthalmology, The University of Illinois; Assistant Professor of Ophthalmology, Northwestern University. Anatomical Transparencies by GLADYS McHUGH, Medical Illustrator, University Clinics, The University of Chicago. Historical Appendix by STEPHEN L. POLYAK, M.D., Professor of Anatomy, The University of Chicago. Pp. 99; 53 black and white illus., 34 colored transparencies. Rochester, N. Y.: Bausch & Lomb Press, 1943. Price, \$6.50 (limited distribution).

*Pain.* Proceedings of the Association for Research in Nervous and Mental Disease, December 18 and 19, 1942. New York. Vol. XXIII. Editorial Board, HAROLD G. WOLFF, M.D., Chairman; HERBERT S. GASSER, M.D., JOSEPH C. HINSEY, Ph.D. Pp. 468; 116 illus.; 19 tables. Baltimore, Md.: The Williams & Wilkins Company, 1943. Price, \$7.50.

*Psychological Medicine.* A Short Introduction to Psychiatry. With an Appendix *War-time Psychiatry.* By DESMOND CURRAN, M.B., F.R.C.P., D.P.M., Psychiatrist and Lecturer in Psychological Medicine, St. George's Hospital, and Honorary Psychiatrist to the Maida Vale Hospital for Nervous Diseases, London, etc.; and ERIC GUTTMANN, M.D., L.R.C.P. (Ed.), Neuropsychiatric Specialist, Emergency Medical Service, etc. Pp. 188; 21 figs. Baltimore, Md.: The Williams & Wilkins Company, 1943. Price, \$3.50.

*The Hospital in Modern Society.* Edited by ARTHUR C. BACHMEYER, M.D., Director, University of Chicago Clinics; Director, Hospital Administration Course, University of Chicago; and GERHARD HARTMAN, Ph.D., Director, Newton Hospital, Newton Lower Falls, Mass. Pp. 768. New York: The Commonwealth Fund, 1943. Price, \$5.00.

*Medical Clinics of North America.* Philadelphia Number (November, 1943). Symposium on Medical Emergencies on the Home Front. Pp. 280. Philadelphia, Pa.: W. B. Saunders Company, 1943. Price, \$16.00 per year.

*Elements of Medical Mycology.* By JACOB HYAMS SWARTZ, M.D., Assistant Professor of Dermatology, Harvard Medical School and the Postgraduate School; Member American Dermatological Association, American Mycological Association; Dermatologist, Massachusetts General Hospital. Foreword by FRED D. WEIDMAN, M.D., Professor of Dermatological Research, University of Pennsylvania. Pp. 190; 80 illus. New York: Grune & Stratton, Inc., 1943. Price, \$4.50.

*Medical Genetics and Eugenics*. Vol. 2. By R. RUGGLES GATES, Ph.D., D.Sc., LL.D., F.R.S., Marine Biological Laboratory, Woods Hole, Mass.; LAURENCE H. SNYDER, B.S., M.S., Sc.D., Professor of Medical Genetics, The Ohio State University; and EARNEST A. HOOTON, Ph.D., Sc.D., Department of Anthropology, Harvard University. Pp. 60. Published by Woman's Medical College of Pennsylvania, Philadelphia, Pa., 1943. Price, \$1.00.

This is the second volume of a series of lectures on Medical Genetics sponsored by an alumna of the Woman's Medical College. It includes 3 lectures given to the public in the spring of 1943 at the College of Physicians of Philadelphia by R. Ruggles Gates (Our Ancestors, Ourselves, Our Descendants); Laurence H. Snyder (Heredity and Modern Life); and Earnest A. Hooton (Human Heredity or Forbidden Fruit of the Tree of Knowledge). The names of the speakers guarantee the excellence of the pabulum provided. The third speaker, as usual, titillates his readers, like his audience, with thought-provoking as well as amusing "Hootonisms." Such a wit should command a high price on the lecture platform, aside from the widening applications and increasing recognition of the importance of human heredity. Curiosity raises the question as to whether the "a" in his first name was given at baptism, or added by its possessor after his literary style became manifest. E. K.

### NEW EDITIONS

*Directory of Biological Laboratories*. With Buyers' Guide. Pp. 96. Published by Burns Compiling & Research Organization, 200 Railway Exchange Bldg., 80 E. Jackson Blvd., Chicago 4, Ill., 1943 (issued annually). Price, \$3.00.

The 1943 edition of this annual publication identifies approximately 800 laboratories concerned with biologic, bacteriologic or biochemical investigations. Research laboratories are included, also commercial consulting laboratories, and those related to manufacturing processes, the latter embracing food and nutrition products, vitamin products, organic chemicals and pharmaceuticals, biologicals, glandular products, and so forth. Personnel and professional data are shown for the laboratories listed. The Buyers' Guide, occupying just one-half of the Directory, gives a useful classified list of sources of supply for apparatus, equipment, instruments, materials, and so on. E. K.

*Pathological Histology*. By ROBERTSON F. OGILVIE, M.D., F.R.C.P. (Ed.), Lecturer in Pathology and Assistant in Forensic Medicine, University of Edinburgh; Senior Pathologist, Royal Infirmary, Edinburgh; Pathologist to the Leith and Deaconess Hospitals, Edinburgh; Examiner in Pathology and Forensic Medicine for the Triple Qualification. Second ed. Pp. 411; 235 photomicrographs in color. Baltimore: The Williams & Wilkins Company, 1943. Price, \$9.00.

*The Foot*. By NORMAN C. LAKE, M.D., M.S., D.Sc. (Lond.), F.R.C.S. (Eng.), Senior Surgeon and Lecturer on Surgery, Charing Cross Hospital; Surgeon Bolingbroke Hospital; Consulting Surgeon, Emergency Medical Service; Director of Studies, London Foot Hospital; etc. Third ed. Pp. 432; 136 figs. Baltimore: The Williams & Wilkins Company, 1943. Price, \$5.00.

*A Synopsis of Surgical Anatomy*. By ALEXANDER LEE MCGREGOR, M.Ch. (Ed.), F.R.C.S. (Eng.), Lecturer on Surgical Anatomy, University of Witwatersrand; Assistant Surgeon, Johannesburg General Hospital. Fifth ed. Pp. 710; 696 illus. Baltimore: The Williams & Wilkins Company, 1943. Price, \$6.50.

*The Dysenteric Disorders*. The Diagnosis and Treatment of Dysentery, Sprue, Colitis and Other Diarrheas in General Practice. By SIR PHILIP MANSON-BAHR, C.M.G., D.S.O., M.D., F.R.C.P., Senior Physician to the Hospital for Tropical Diseases, Royal Albert Dock and Tilbury Hospitals; Consulting Physician in Tropical Diseases to the Dreadnaught Seamen's Hospital, London; Director, Division of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine; Consulting Physician to the Colonial Office and Crown Agents for the Colonies; Consultant in Tropical Medicine to the Admiralty and to the Royal Air Force; etc. Second ed. Appendix by W. JOHN MUGGLETON, M.S.M., F.I.M.L.T., Technical Assistant. Pp. 629; 108 illus. (9 colored). Baltimore: The Williams & Wilkins Company, 1943. Price, \$10.00.

*Symptoms and Signs in Clinical Medicine.* An Introduction to Medical Diagnosis. By E. NOBLE CHAMBERLAIN, M.D., M.Sc., F.R.C.P., Lecturer in Medicine, University of Liverpool; Physician to Out-patients, Royal Liverpool United Hospital, Royal Infirmary Branch; Visiting Physician, Smithdown Road Hospital, Liverpool. Third ed. Pp. 456; 346 illus. (19 colored). Baltimore: The Williams & Wilkins Company, 1943. Price, \$8.00.

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### NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

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Activated by a directive from the War Production Board, we have changed the size of our type page for the "duration" to effect an economy in the amount of paper used. While there is a smaller number of pages, the amount of material has not been noticeably reduced.

We hope that any unpleasant effect produced by cutting down the margins will be accepted and approved by readers as a temporary war casualty. It is possible that more radical changes will have to be made later, but we are loath to change any more than absolutely necessary, a format that has existed practically unchanged since the Journal began in 1820.

For the balance of the war, 150 reprints will be supplied gratis. Covers will be omitted on all articles. In ordering additional reprints, we will supply in multiples of 150. This modification is for the same reason as the change of format, *i. e.*, conservation of paper.

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We desire to secure several copies of the March and May 1943 numbers of this Journal, in order to comply with requests and need for replacements in long library "runs." The war situation has made it impossible to print extra numbers to supply this demand. We would be *very* grateful to anyone who would return to the Publishers any unutilized copies of these numbers for which they have no further use, and we would be glad to repay postage.

# THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

FEBRUARY, 1944

## ORIGINAL ARTICLES

### OSTEOSCLEROSIS, MYELOFIBROSIS AND LEUKEMIA

BY JACOB CHURG, M.D.

GEORGE BLUMENTHAL, JR., FELLOW IN PATHOLOGY

AND

MAX WACHSTEIN, M.D.

FELLOW OF THE DAZIAN FOUNDATION FOR MEDICAL RESEARCH  
NEW YORK, N. Y.

(From the Laboratories of the Mt. Sinai Hospital)

OSTEODYSTROPHIC changes in the bones such as osteosclerosis and fibrosis of bone marrow may be accompanied by a disturbance of the white blood cell picture, characterized by a high count and the appearance of immature elements in the circulation, as well as by leukopenia and anemia. Because of the blood changes, as well as splenomegaly and progressive fatal course, the first observers<sup>2,7,18,19</sup> regarded such cases as instances of true leukemia, or at least, very closely allied with the latter condition. "Osteosclerotic leukemia" merits a special section in Henke-Lubarsch's Handbook of Pathology.<sup>6</sup> However, more recently, there has appeared a tendency to separate all these cases from leukemia and to place them in a different group, known under a variety of names, such as chronic non-leukemic myelosis,<sup>14</sup> megakaryocytic myeloid splenomegaly,<sup>4</sup> leuko-erythroblastic anemia,<sup>21</sup> and so on. The non-leukemic myelosis is similar to leukemia in many of its clinical and pathologic aspects, but has been adequately differentiated from the latter condition and established as an independent entity by recent investigations.<sup>3,8,10</sup> As all reports indicate<sup>3,10,20</sup> the non-leukemic myelosis is frequently associated with osteodystrophic changes. It seems important, however, to determine, especially from the prognostic standpoint, whether such changes do not also occur in leukemia. For that purpose, we have reexamined, paying special attention to the changes in the bone marrow, all cases in our laboratory which in the last 10 years were diagnosed as leukemia on postmortem examination. It must be mentioned, however, that possible instances of non-leukemic myelosis might have been included under this heading.

Among our material, there were 82 cases of myeloid leukemia—46 of the acute or subacute, and 36 of the chronic variety, as well as 15 cases of lymphatic leukemia. Of these 97, 7 showed considerable or even pronounced osteodystrophic changes—2 in the subacute myeloid

group and 5 in the chronic myeloid. The subacute cases did not receive any specific therapy, whereas the chronic cases were subjected to radiation in varying amounts. Only 2 cases of the latter group are presented below; the other 3 showed changes very similar to those found in Case 3, and are therefore omitted for the sake of brevity.

**Case Studies.** CASE 1. J. D., white, male, age 18; No. 486191 (P. M. 12128). The patient was admitted to the Mount Sinai Hospital in July 1941, complaining of severe low back pain and fever. He was well developed for his age. There were no significant physical findings except for a moderate generalized enlargement of the lymph nodes. The blood count showed: Hgb. 80%, R.B.C. 4.12 mill.; W.B.C. 4650 (neutrophils 56%, lymphocytes 42%, monocytes 2%), no abnormal cells in the smear. Enteric fever and infectious mononucleosis were considered, but the temperature dropped promptly, the pain subsided, and the patient was discharged with the diagnosis of the gripe. He was readmitted in 5 weeks with pain in the left chest and fever. The enlargement of the lymph nodes was more prominent, the spleen and liver became palpable while under observation. The blood count revealed: Hgb. 62%, R.B.C. 3.07 mill., W.B.C. 4050 (myeloblasts 8%, non-segmented neutrophils 11%, segmented 20%, lymphocytes 54%, monocytes 4%, eosinophils 2%, plasma cells 1%, normoblasts 2 per 100 W.B.C.), platelets 40,000. Sternal aspiration showed an almost completely myeloblastic bone marrow, thus establishing the diagnosis of myeloblastic leukemia. Roentgen ray examination of the ribs was entirely negative. The Wassermann reaction gave negative results on repeated occasions.

From September 1941 until the time of his death in March 1942, the patient was under observation in the hospital and in the hematology clinic. His white count always remained low, sometimes dropping to 700 and never rising above 13,000. In October 1941 the blood count was as follows: Hgb. 58%, R.B.C. 3.0 mill., W.B.C. 7300 (myeloblasts 44%, promyeloblasts 1%, myelocytes 3%, non-segmented neutrophils 5%, segmented 4%, lymphocytes 36%, monocytes 3%, eosinophils 1%, 6 normoblasts and 1 erythroblast per 100 W.B.C.). The patient was sustained by repeated blood transfusions, yet his hemoglobin continued to drop, especially during the numerous febrile episodes. He developed left purulent otitis media and mastoiditis which were temporarily held in check by sulfonamides, but eventually flared up and apparently became the starting point of the terminal sepsis. On his last admission his Hgb. was 23%, W.B.C. 800 (20% myeloblasts, 1% myelocytes, 2% non-segmented neutrophils, 14% segmented, 62% lymphocytes and 1% monocytes). In spite of sulfathiazole administration, the temperature rose to 106° F. and the patient died 8 months after the onset of first symptoms.

**Autopsy** (5 hours after death). Only pertinent findings are recorded. The body was pale and emaciated. Numerous petechiae were found over the trunk and extremities; the gums were covered with clotted blood.

**Heart:** Dilated, very flabby and pale. The myocardium showed diffuse yellowish mottling caused by focal fatty changes in the muscle. *Microscopic* collections of myeloid cells were found within the epicardium.

**Lungs** contained scattered and confluent areas of bronchopneumonia, especially numerous in both lower lobes. These areas were slightly raised, dry, and varied in color from gray to dark red. Several pinhead-size abscesses were found in the right upper lobe. The hilar lymph nodes were moderately enlarged, soft and anthracotic. *Microscopically*, the alveoli within the pneumonic areas were filled with fibrin together with a varying admixture of red cells and young myeloid cells but very few polymorphonuclears. There were many small focal collections of myeloid cells around the bronchi.

**Liver** weighed 2360 gm. The capsule was smooth and brown in color. Under the capsule and throughout the parenchyma, there were scattered numerous pinhead-sized abscesses filled with creamy yellowish pus. The lobular architecture of the liver was blurred, though there were many grayish dots and

streaks which seemed to correspond with the periportal areas. *Microscopically*, there was distinct cellular infiltration of the periportal fields, many of which were considerably enlarged. In addition to lymphocytes, there were also numerous, young myeloid cells (myeloblasts and myelocytes). The sinusoids were congested, but otherwise not involved. The Kupffer cells were prominent. The abscesses were often surrounded by collections of myeloid cells and small cells with dark nuclei and scanty cytoplasm, either lymphocytes or atypical myeloblasts. There were no megakaryocytes or erythroblasts. Numerous granules of hemosiderin were found both within and outside of the liver cells.

*Spleen:* Large (weight 600 gm.). Several yellowish white infarcts were observed near the upper pole. On section, the cut surface was moderately firm and dark red in color. The trabeculae were distinct. The Malpighian corpuscles were fairly numerous but very small. Under the *microscope* it could be seen that the architecture was rather well preserved, though both the red and the white pulp were infiltrated with very young myeloid cells. The infiltration was focal rather than diffuse and especially prominent in the perifollicular zones. Often the foci of infiltration consisted of small groups of large, very immature cells with large vesicular nuclei surrounded by smaller myeloblasts and myelocytes. In addition to the myeloid cells there was a fair number of scattered megakaryocytes and small nests of erythroblasts. Hemosiderin was found in large quantities, both intracellular and extracellular.

*Lymph nodes*, including the perinotic, peripancreatic, portal and mesenteric, were large measuring up to 2 cm. in diameter. They were soft in consistency and pinkish red in color. The cut surface had a homogenous reddish gray appearance. *Microscopically*, all of the examined lymph nodes showed a diffuse myeloid metaphasia with more or less pronounced obliteration of the normal architecture. There were no erythroblasts or megakaryocytes.

*Bone Marrow:* Because of the limited permission, only the vertebral column was examined. The bony trabeculation was distinct throughout. The marrow was red and moist but in many areas it appeared pale, dry, and grayish in color. *Microscopic* sections at different levels showed a somewhat varying picture (Figs. 1 and 2). In general, there was a widening of the trabeculae, but the intertrabecular spaces did not appear to be narrowed. The normal marrow had completely disappeared. In most of the sections it was replaced by very loose connective tissue surrounding wide vascular spaces. Within the meshes of the connective tissue, there was found a small number of myeloid cells, occasional erythroblasts and, very rarely, megakaryocytes. In places, the meshes were filled with pinkish stained homogenous material. In other sections, the process of marrow replacement appeared to have progressed further than in the previous ones. The vascular spaces were less prominent, the connective tissue much denser, the bone marrow cells almost totally absent. In these areas there was also formation of new bone trabeculae.

Focal collections of myeloid cells were also found in the *kidneys* and the *pancreas*. The *cecum* showed two small ulcerations surrounded by lymphocytes, myeloid cells, and very few neutrophils. *Testes* were small and underdeveloped; the tubules were separated by a large amount of cellular connective tissue, and showed no spermatogenesis.

**CASE 2.** L. S., white, male, age 67; No. 411030 (P. M. 10438). Except for a "nervous breakdown" a year before admission, the patient was always well. For the past 6 months he noted frequent appearance of "black and blue marks" without any obvious cause. This was accompanied by increasing fatigability and palpitation of the heart. A mild attack of cough about 2 weeks prior to admission was followed by persistent severe left-sided headache, repeated nosebleeds and marked weakness with night sweats. On admission, the temperature was 101° F., pulse 120. There were numerous purpuric spots over both elbows. The fundi showed flame-shaped and punctate hemorrhages. A sloughing ulcer and multiple bleeding points were found on the gums. The heart was enlarged, with apical and basal systolic murmur. The spleen reached 3 fingerbreadths and the liver 1 fingerbreadth below the costal arches. There was a pitting edema of both ankles. The subcutaneous lymph nodes were not

conspicuously enlarged. The blood count showed: Hgb. 34%, R.B.C. 1.3 mill., W.B.C. 8500 (31% segmented neutrophils and 2% non-segmented, 4% monocytes, 63% indetermined mononuclear cells and 2 normoblasts per 100 nucleated cells); platelets 80,000. A bone marrow biopsy showed replacement

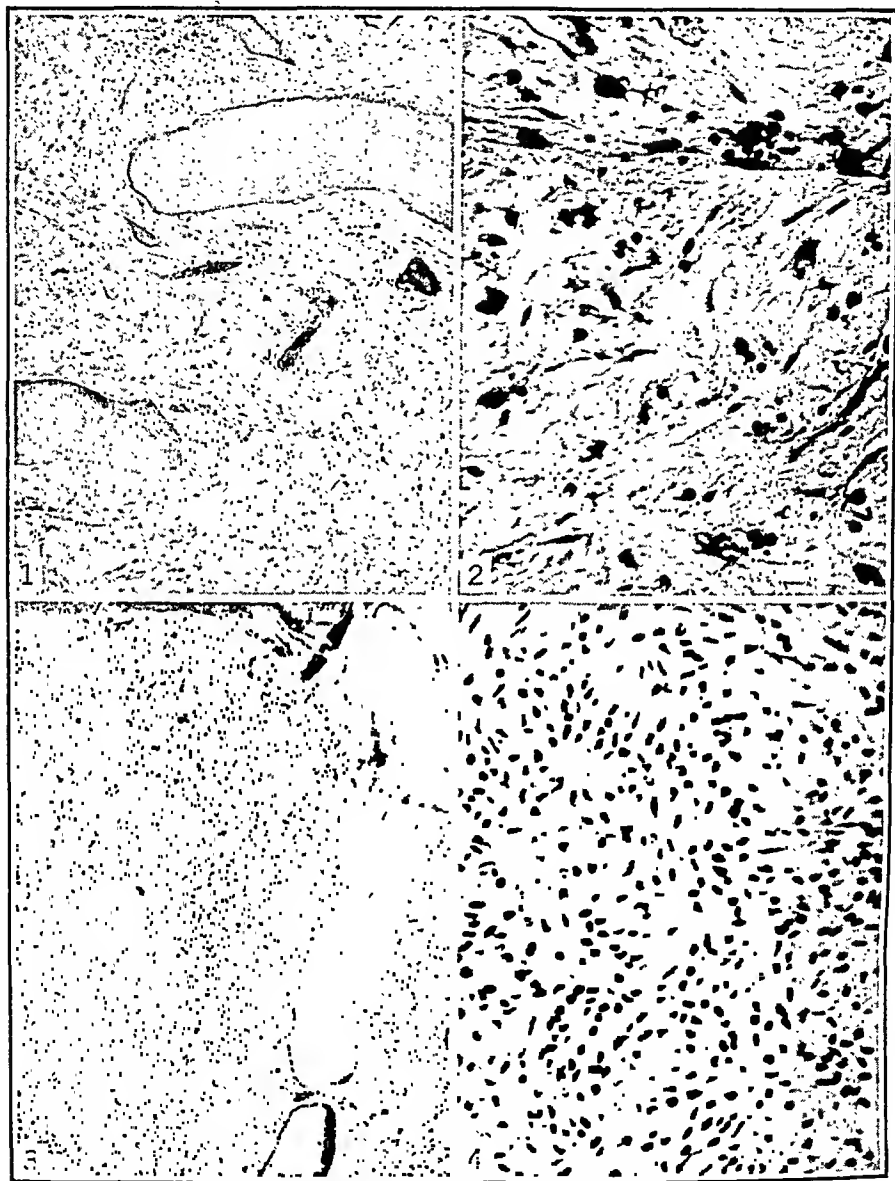


FIG. 1.—Case 1. *Bone marrow, subacute myeloid leukemia.* Replacement of the bone marrow by loose connective tissue and a few newly-formed bony trabeculae. 75X.

FIG. 2.—Case 1. *Bone marrow.* Note the scattered myeloid cells. 360X.

FIG. 3.—Case 2. *Bone marrow, subacute myeloid leukemia.* Replacement of the normal marrow by cellular connective tissue and numerous myeloid cells. 75X.

FIG. 4.—Case 2. *Bone marrow.* 360X.

of the normal marrow by young connective tissue and young myeloid cells in a manner similar to that seen at autopsy. In spite of several large transfusions, the patient went rapidly downhill. His temperature rose to 104° F. in the next 4 days and remained at that level until his death, 9 days after admission.

**Autopsy** (2½ hours after death). There were scattered small petechial spots over the chest and over both arms and thighs. The gums appeared spongy and showed ulceration at the base of the lower incisors. Small petechial hemorrhages were found throughout the gastro-intestinal tract.

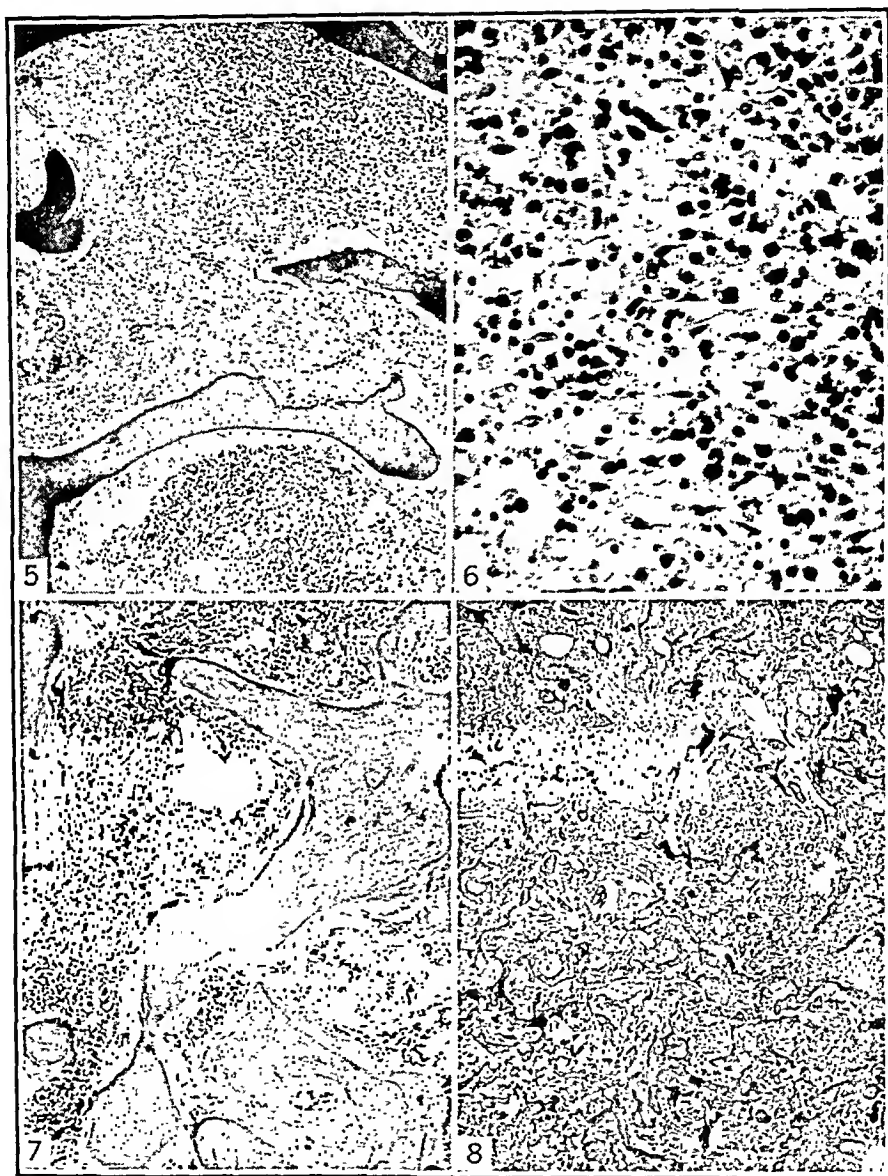


FIG. 5.—Case 3. *Bone marrow, chronic myeloid leukemia* treated with radiation. Fibrosis with islands of leukemic myeloid cells. 75X.

FIG. 6.—Case 3. *Bone marrow.* 360X.

FIG. 7.—Case 4. *Bone marrow, chronic non-leukemic myelosis.* Marked osteosclerosis and partial fibrosis. Note the enormous number of newly-formed bone trabeculae and large areas of cellular marrow of normal composition. 95X.

FIG. 8.—Case 4. *Bone marrow.* 15X.

**Heart:** Enlarged, weighing 500 gm. The pericardial sac contained about 75 cc. of clear, serous fluid. There were a number of petechial hemorrhages in the parietal pericardium. A few petechiae were also found under the endo-



cardium of the right and left auricle. The right ventricle was moderately, and the left ventricle markedly hypertrophied. All chambers of the heart were dilated. The myocardium of both ventricles was flabby, grayish brown in color, with areas of yellowish streaking.

*Lungs:* Over the posterior aspect of the right lung there was a continuous hemorrhagic area, 12 by 3 cm., extending for about 0.5 cm. into the parenchyma. Otherwise the lungs showed evidence of emphysema and, microscopically, focal infiltration of alveolar walls by young myeloid cells.

*Liver* (weight 2500 gm.) was enlarged and flabby. The cut section presented a distinct lobular architecture. The periportal fields were wide, brownish yellow and slightly elevated. The central zones were reddish brown and depressed. Under the microscope, the periportal fields were rather densely infiltrated, while the sinusoids contained only a few scattered abnormal cells. The infiltration consisted partly of large cells with large clear nuclei, partly of small cells with dark nuclei and scanty cytoplasm. Only a few maturing white elements were seen. There were no erythroblasts or megakaryocytes.

*Spleen* (weight 1800 gm.) was markedly enlarged and firm in consistency. The surface was purplish blue with irregular grayish white thickening of the capsule. The cut surface was light red in color, with irregular but well demarcated darker red areas, from pinhead to 2 cm. in diameter, scattered throughout the pulp. *Microscopically*, the spleen was densely infiltrated by cells similar to those found in the liver. Only a few of these cells gave a positive oxydase reaction. The Malpighian corpuscles could not be recognized. There were no foci of extramedullary blood formation.

*Kidneys* had a faintly granular surface. On microscopic examination they showed small scattered nests of myeloid cells.

*Lymph Nodes:* The abdominal and thoracic nodes were enlarged and measured up to 2 cm. in diameter. The cut surface was pinkish in color with irregular grayish areas. *Microscopically*, the normal architecture was preserved in places, but mostly obliterated by diffuse infiltration of myeloid cells. There were no erythroblasts or megakaryocytes.

*Bone Marrow:* Sections through the lumbar vertebræ showed the usual bony trabeculation. The marrow was grayish red with scattered lighter yellowish areas. The sternal marrow was likewise grayish red and dry. Under the *microscope* (Figs. 3 and 4) the normal marrow had almost completely disappeared. The marrow spaces were filled with young myeloid cells, congested vascular channels and young connective tissue, in varying proportions. With the Mallory stain, the connective tissue fibers formed a dense network between and around the myeloid cells, and in places, aggregated into larger fibrous areas. A few islands of fatty marrow were also present.

CASE 3. M. T., white, male, age 47, No. 482527 (P. M. 12048). The patient was in apparent good health until the end of November 1940, when the present illness began with fainting spells and passing of tarry stools. Gastric ulcer was suspected but not confirmed on Roentgen ray examination. At that time an enlarged spleen was noted in Roentgen ray films. Because of progressive loss of strength and weight the patient was hospitalized in March 1941. The physical examination revealed enlargement of the liver and spleen, the latter being the size of a grapefruit, and the former reaching 3 fingerbreadths below the costal arch. There were no palpable peripheral lymph nodes. Stool gave a positive test for occult blood. Repeated blood counts showed: Hgb. 80%, R.B.C. 4.7 to 4.85 mill., W.B.C. 79,600 to 132,000 (segmented neutrophils 37%, non-segmented 9%, metamyelocytes 9%, myelocytes 10%, promyelocytes 10%, myeloblasts 7%, eosinophils 3%, basophils 7%, lymphocytes 8%). Aspiration of sternal marrow was performed and showed: myeloblasts 11%, neutrophilic myelocytes 56.5%, eosinophilic 4%, basophilic 1.5%, neutrophils 27%; there was only 1 erythroblastic cell per 100 white cells. Diagnosis of myeloid leukemia was made and the patient was discharged after 1 Roentgen ray treatment to the left flank. In June 1941 a series of 6 Roentgen ray treatments to the spleen was administered in another institution (3 times a week, 100 rx3, anteriorly and posteriorly). The blood count before the treatment

was Hgb. 88%, R.B.C. 4.32 mill., W.B.C. 131,200 (myelocytes 4%, metamyelocytes 7%, neutrophils 70%, basophils 3%, monocytes 1%, lymphocytes 15%, nucleated red cells 4 per 100 W.B.C.). The white blood count dropped rapidly and at the conclusion of the irradiation series was below 10,000. Three weeks after the treatment the blood count was Hgb. 65%, R.B.C. 3.39 mill., W.B.C. 5000 (neutrophils 75%, monocytes 3%, lymphocytes 22%). The drop in the red blood cells and white blood cells persisted on all subsequent examinations.

At the time of admission (September, 1941), the liver reached 3 fingers and the spleen 6 fingers below the costal margin. There was no adenopathy. A flame-shaped hemorrhage was present in the fundus of the left eye. Blood count showed: Hgb. 64%, R.B.C. 3.36 mill., W.B.C. 1400 (myeloblasts 2%, myelocytes 10%, non-segmented 17%, segmented neutrophils 46%, basophils 1%, monocytes 2%, lymphocytes 25%, plasma cells 1%, normoblasts 1 per 100 W.B.C.; platelets 50,000; *Roentgen ray examination of the bones* revealed "several small osteoporotic areas in the distal end of the right femur, just above the condylar region, and in the proximal half inch of the right tibia. There is also a suggestion of osteoporosis of the descending ramus of the right pubic bone. The significance of these findings is not determined. In view of the clinical findings of leukemia, these are probably due to leukemic infiltrations. There is no periosteal elevation."

During his stay in the hospital, the patient developed a transient jaundice. Despite a temporary improvement after transfusions, the course was rapidly downhill. On his second admission in December 1941, the patient had fever and ascites. Both the liver and spleen were enormously enlarged, reaching down to the iliac crests. Blood count showed: Hgb. 58%, R.B.C. 3.2 mill., W.B.C. 7800 (myeloblasts 30%, neutrophilic myelocytes 3%, basophilic 1%, non-segmented neutrophils 12%, segmented 39%, monocytes 1%, lymphocytes 12%, plasma cells 2%); platelets 5000. The sternal marrow (aspiration) was predominantly myeloblastic as seen in leukemia. Patient became progressively more drowsy and died in pulmonary edema 13 months after the appearance of the first symptoms.

**Autopsy** (2 hours after death). *Liver*: Markedly enlarged (weight 2800 gm.). The consistency was softer than normal and the edge was rounded. Under the transparent capsule and throughout the liver on cut section, there were scattered numerous discrete and confluent, slightly depressed dark red areas, irregular in outline and varying in size from that of a pinhead to 2 cm. in diameter. These areas were more numerous in the left lobe and more prominent in the subcapsular region. The intervening liver parenchyma was yellowish brown with an occasional pin-point grayish area. The lobular structure was fairly distinct. On *microscopic examination* it could be seen that the dark red areas were caused by extensive focal hemorrhages with necrosis of liver cells. Otherwise the parenchyma appeared fairly normal except for a moderate degree of fatty infiltration. The sinusoids were dilated and contained myeloid cells, single and in small groups. Though the predominant cell was the myeloblast, myelocytes were also seen. There were many cells in mitosis. The Kupffer cells were prominent. The portal fields contained occasional small collections of lymphocytes, but were entirely free of leukemic cell infiltration. Nowhere were there any signs of extramedullary hemopoiesis.

*Spleen* weighed 880 gm. and was moderately firm. The surface was smooth and dark bluish purple. The cut surface presented a homogeneous fleshy reddish brown appearance; only the trabeculae but not the follicles could be made out. A cherry-size accessory spleen of a similar structure was found near the hilus. *Microscopic examination*: The Malpighian corpuscles were very small. The section had a rather homogeneous appearance caused by a diffuse infiltration of the pulp by myeloid cells. The infiltration was moderately intense so that it did not obscure the vascular pattern of the red pulp. The sinusoids were clearly discernible; many of them contained small groups of myeloid cells. The amount of fibrous tissue was definitely increased throughout the spleen. Silver stain brought out the dense network of reticulum fibers mainly in the peritrabecular zones. Sections of the accessory spleen showed

less fibrosis but a more pronounced infiltration by myeloid cells. There were no foci of extramedullary blood formation in the spleen or in the accessory spleen.

**Lymph Nodes:** The periaortic, peripancreatic and portal lymph nodes were enlarged, grayish in color and soft in consistency. The cut surface appeared moist and cellular. *Microscopically*, all of the examined lymph nodes showed leukemic involvement of varying degree. In those most markedly involved, the normal architecture was considerably obliterated, though a few lymph follicles could still be seen beneath the capsule. The cortex between the follicles as well as the medulla were infiltrated by myeloid cells. The infiltration was diffuse, but not very dense, so that a considerable number of lymphocytes could still be seen between the myeloid cells. A number of giant cells with pink cytoplasm and multilobulated nuclei were scattered throughout the lymph nodes. Some of these cells bore a close resemblance to megakaryocytes. There were no erythroblastic elements. In other lymph nodes the leukemic infiltration was less intense and was limited mainly to the medulla, leaving the architecture practically undisturbed.

**Bone Marrow:** Because of the limited permission, examination was confined to the lumbar vertebrae. On section, the bony trabeculation appeared normal, while the marrow between the trabeculae was grayish, rather firm and dry. On *microscopic examination* (Figs. 5 and 6) the bony trabeculae were normal in width and number. The cellular as well as the fatty marrow were completely replaced by a network composed of fibroblasts and delicate fibers which assumed blue color with the Mallory collagen stain and black color after silver impregnation. The meshes of this network contained a fair number of leukemic myeloid elements together with occasional nests of erythroblastic cells and very few megakaryocytes.

All other organs showed no significant changes and no leukemic infiltrations. The lungs presented a healed primary tuberculous focus with calcified tracheal lymph nodes.

**CASE 4.** M. D., white, female, age 60; No. 294186 (P. M. 6365). The patient was first seen at the Mount Sinai Hospital in 1914, when she underwent hysterectomy for uterine fibromyomata. At that time there was apparently no splenomegaly or hepatomegaly. In 1920, she was readmitted because of severe pain in the left upper quadrant, loss of appetite and weakness. The spleen and liver were markedly enlarged, the former descending down to the umbilicus and being tender to palpation, and the latter reaching 3 fingers below the costal arch. The inguinal lymph nodes were slightly enlarged but there was no generalized lymphadenopathy. Blood count showed: Hgb. 72%, R.B.C. 4.17 mill., W.B.C. 9800 (neutrophils 68%, eosinophils 0.5%, basophils 0.5%, myelocytes 13.5%, myeloblasts 5%, lymphocytes 10%, monocytes 2.5%, a few normoblasts in the smear); platelets 250,000. On that basis the diagnosis of leukemia was accepted as most likely.

After discharge from the hospital, the patient was observed in the hematology clinic until 1928. She was given Roentgen ray treatments, though with little success. Her liver and spleen continued to enlarge, the latter frequently being very tender. The general condition deteriorated, at first slowly and then rapidly. Hgb. and R.B.C. gradually dropped to 50% and 3.2 mill., respectively; W.B.C. varied between 8200 and 39,000 (50 to 60% neutrophils, 10 to 20% myelocytes and 8 to 11% myeloblasts). The platelet count always remained within the normal limits, around 200,000 to 250,000. Because of weakness and marked emaciation, she was again admitted to the hospital in August 1928. Despite transfusions, her anemia rapidly progressed. W.B.C. remained as previously, though on one occasion it rose to 125,000 (80.5% neutrophils, 10.5% myelocytes, 3% myeloblasts, 3.5% lymphocytes, 1% monocytes and 19 normoblasts per 100 W.B.C.). The patient developed pneumonia which, together with increasing cachexia, led to her death 3 weeks after admission. The total duration of her illness was over 8 years.

**Autopsy** (11 hours after death). **Heart:** Moderately enlarged. The coronary arteries were sclerotic. The myocardium showed scattered areas of fibrosis

and fatty changes in the muscle fibers. The capillaries were dilated and contained many myeloid cells, both young forms and polymorphonuclears. Under the epicardium there were small focal collections of similar cells.

*Lungs:* Both lower lobes contained diffuse areas of bronchopneumonia. These showed abundant fibrinous exudate and numerous polymorphonuclear leukocytes as well as young myeloid cells. Numerous giant cells with dark pleomorphic nuclei were found in all microscopic sections. Focal infiltrations by myeloid cells were seen in the peribronchial and perivascular areas.

*Liver* (weight 1800 gm). The right lobe was elongated and enlarged. The surface was smooth. On section the parenchyma presented a waxy reddish appearance with occasional grayish streaks. On *microscopic examination*, the sinusoids were dilated and filled with myeloid cells in all stages of development. There were also occasional giant cells, some of which resembled megakaryocytes. The Kupffer cells were very prominent. The liver cells, especially in the center of the lobules, contained fat droplets and hemosiderin. The periportal fields were free from infiltration. There were no erythroblastic elements.

*Spleen:* Markedly enlarged and weighed 660 gm. It was firm to touch. The surface was smooth and bluish gray in color with numerous scattered white areas of infarction. The splenic pulp was pinkish gray and did not scrape off easily. The Malpighian corpuscles were not visible. The microscope revealed a marked disturbance of the normal architecture. The Malpighian corpuscles had completely disappeared. The sinusoids of the red pulp were prominent but appeared to be widely separated by thickened Bilot's cords. The latter showed a conspicuous increase in reticulum cells, fibroblasts and connective tissue fibers forming a fine network. The meshes of this network contained nests of myeloid cells in various stages of development, as well as small collections of erythroblastic cells. There was also a considerable number of megakaryocytes. The sinusoids were for the most part empty, though some of them contained red blood cells and small collections of myeloid cells. The endothelial lining of the sinusoids was very prominent.

*Kidneys:* The right kidney was larger than the left. The capsules stripped with great difficulty revealing irregular surfaces with several protruding small nodules. These were firm in consistency and grayish white in color. The pelvis of the right kidney contained numerous sand-like brownish calculi; similar calculi were found in the bladder. *Microscopically*, the grayish nodules are found to be small mesodermal tumors, composed of fibrous, fatty and myxomatous tissue. Scattered through the parenchyma of both kidneys were small foci of myeloid cells in various stages of development. A few giant cells of the megakaryocytic type were found in the capillaries and in the glomerular loops.

*Gastro-intestinal Tract:* The ileum and the colon presented several small superficial reddish gray areas of necrosis. The underlying submucosa and muscularis were infiltrated with neutrophils.

*Lymph Nodes:* The mesenteric and the retroperitoneal lymph nodes were moderately enlarged and rather firm in consistency. *Microscopically*, they showed a varying degree of myeloid metaplasia, with loss of normal structure. In some of the nodes the infiltration consisted almost exclusively of myeloid cells in various stages of development; in others, there were in addition prominent nests of erythroblasts and very numerous cells of the megakaryocytic type.

*Bone Marrow:* The tibia was opened for a short distance. Its marrow cavity was filled with spongy bone. Similarly, the marrow cavity of the lower third of the humerus was completely filled with spongy bone. The lumbar vertebrae had, on section, a dense spongy appearance and were pink in color. *Microscopic examination* (Figs. 7 and 8) showed a marked increase in the number of bony trabeculae which were wide and tortuous and varied in their calcium content. There was also a striking formation of new bone and marked narrowing of marrow spaces. The latter were often frequently completely filled by fibrous tissue. Other areas of the bone marrow, in spite of the increase in connective tissue, were very cellular and contained numerous myeloid elements, erythroblastic cells and megakaryocytes in increased numbers.

**Comment:** The first 3 cases as can be seen from the descriptions (and the 3 not extensively reported here) are unequivocal examples of leukemia, according to the most generally accepted standards. They obviously fulfill the criteria as far as the clinical course, the blood picture and the pathologic findings are concerned.\* The occurrence of foci of extramedullary blood formation in Case 1, and of giant cells in the lymph nodes in Case 3, is in accord with the leukemia concept.<sup>16,17</sup>

The fourth case presents a different picture. Clinically it was observed for 8 years and classified as chronic myeloid leukemia. The white blood count was elevated, reaching 125,000 on one occasion. Numerous immature white cells and nucleated red cells were regularly found in the circulating blood. The bones, including the long bones, exhibited osteosclerosis with areas of complete myelofibrosis, as well as areas of hyperplastic bone marrow. There were extensive infiltrations in the liver, spleen, lymph nodes and also small foci in pericardium and kidneys; the infiltrations consisted of myeloid cells in various stages of development, and of numerous megakaryocytes and erythroblasts, the latter being prominent in the lymph nodes and spleen. The same diversity without the customary preponderance of young white cells was observed in the hyperplastic foci of bone marrow. The long clinical course; the moderately elevated white blood cell count with a low proportion of primitive cells; the constant presence of nucleated red blood cells; the chronic splenomegaly; the absence of typical leukemic infiltrations, and the diversity of cells forming the infiltrations in the various organs with an unusually large number of megakaryocytes—all tend to place this case in the leukemoid<sup>12</sup> group of non-leukemic myelosis.

The changes in the bone marrow of the first 3 cases were of an essentially similar type. The available sections of bone (unfortunately no long bones could be examined because of the limited autopsy permissions) showed a varying degree of fibrosis. In Case 2 the marrow was cellular and characteristically leukemic; fibrosis was represented by a meshwork of fine threads of connective tissue; there was no appreciable change in the bony trabeculation. In Case 1, on the other hand, the bony trabeculation was somewhat denser than normal with occasional areas of newly formed bone. The hematic cells had all but completely disappeared from the marrow which was almost made entirely up of young mucoid, rather cellular connective tissue. Neither of these cases had received any Roentgen ray treatment. Case 3 presented similarly marked fibrosis, with still clearly discernible islands of leukemic myeloid cells, but without any definite increase in the bony trabeculation. This case belongs to the group of chronic myeloid leukemia and was treated with small doses of Roentgen ray, so that fibrosis can be attributed, at least in part, to the latter factor. However, it has to be pointed out that in our material only 4 out of 50

\* The diagnostic criteria for the leukemias still are of a cumulative nature, rather than any one being pathognomonic. It is well recognized that a number of conditions can produce leukemoid<sup>12</sup> blood pictures as well as clinical signs and symptoms, and even the characteristic histologic picture can be simulated. One must be most careful, therefore, in the use of unreserved allocations.—EDITOR.

cases of chronic leukemia, showed fibrosis of the bone marrow though all of them received the standard treatment of this disease. To summarize, the osteodystrophic changes in the 6 cases of leukemia consisted of myelofibrosis—an increase in connective tissue within the marrow with replacement of the cellular elements. There was little or no formation of new bony trabeculae and, therefore, the marrow spaces were not narrowed to any appreciable extent.

The appearance of the bone marrow in the fourth case, here reported—the non-leukemic myelosis—was dominated by a great amount of newly formed bone with marked narrowing of the marrow spaces. The latter were partly filled by connective tissue and partly by hyperplastic marrow. The trabeculae were wide and tortuous and showed a varying degree of calcification. This is the typical picture of *osteosclerosis*, as defined by Kaufmann,<sup>11</sup> it is “the antithesis of osteoporosis and consists of the formation of new, at first osteoid, then calcified true bone, coming from the marrow and vessel cavities and encroaching upon the old trabeculae. The spaces within the bone are filled with more and more bone tissue.” Osteosclerosis is commonly accompanied by a varying degree of fibrosis of bone marrow, but, as demonstrated above, myelofibrosis occurs also without osteosclerosis.

The literature contains only a few examples of leukemia associated with myelofibrosis, though apparently it is not very rare. The case published by Lehndorff and Zak<sup>13</sup> is often called osteosclerotic leukemia, but in reality is a typical instance of myelofibrosis without a trace of osteosclerosis. Mettier and Rusk<sup>15</sup> reported 2 cases under the title: “Fibrosis of the bone marrow (myelofibrosis) associated with leukemoid blood picture.” The case of Anagnostu<sup>1</sup> might also belong in this group.

Reports of “osteosclerotic leukemia” are more numerous. However, we agree with Carpenter and Flory<sup>3</sup> that the great majority of cases of osteosclerosis with a leukemic or leukemoid blood picture can be classified as non-leukemic myelosis. We were able to find only 2 cases which do not fulfill the criteria of non-leukemic myelosis and can be regarded as instances of leukemia, though by no means typical. The case of Jacobson<sup>9</sup> is reported very briefly and the details are lacking. It concerns a 28 year old chemist, who worked with pyridine and aniline dyes and developed a marked anemia and leukopenia with many atypical cells in the circulation. The postmortem examination revealed extensive osteosclerosis and typical leukemic infiltrations in the liver, spleen, kidneys and intestines. In the case of Haessler and Krauspe,<sup>5</sup> a girl aged 2 years and 8 months suffered from increasing anemia. The leukocytes varied between 2600 and 7200. No typical leukemic cells were found in the blood. The duration of the disease was 6 months. At autopsy there was marked generalized osteosclerosis with foci of typical leukemic bone marrow and extensive infiltration of many organs by leukemic cells with localized tumor formation. The existence of such cases points to the need of careful and unbiased examination in every instance of osteosclerosis associated with blood changes.

**Summary and Conclusions.** 1. Among 97 cases of leukemia, 6 showed a varying degree of myelofibrosis without osteosclerosis. Four of these cases were of the chronic myeloid type and were treated with Roentgen ray; the other 2 were diagnosed as subacute myeloid leukemia and did not receive any Roentgen ray therapy.

2. Myelofibrosis, not associated with osteosclerosis, is apparently not uncommon in the leukemias.

3. A case of osteosclerosis and leukemoid blood picture is described and interpreted as an instance of non-leukemic myelosis.

4. A review of the literature shows that osteosclerosis is often associated with non-leukemic myelosis and only very rarely, if at all, with true leukemia.

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### DIAGNOSTIC PHYSICOCHEMICAL BLOOD TESTS IN SICKLE CELL ANEMIA

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IN a recent study of the sedimentation rate in sickle cell anemia, it was noted that the sedimentation of the erythrocytes had certain characteristics which were not encountered in any other normal or disease states.<sup>3</sup> These features have a diagnostic value and may serve to supplement the other diagnostic procedures commonly employed.<sup>1,2</sup>

These characteristics may be organized and used as clinical diagnostic tests. Two separate tests are described, although they are based upon the same principles, *viz.*, the increase of the sedimentation rate of the erythrocyte of sickle anemia by  $O_2$  and the reduction of the sedimentation rate by  $CO_2$ . The tests differ, therefore, only in the methods by which these changes are brought about. These tests are described since they emphasize certain physicochemical peculiarities of the blood in sickle cell anemia, and offer themselves as objective procedures for the diagnostic study of sickle cell anemia. They have certain definite advantages. First, they do not depend solely upon the sickling phenomenon for the diagnosis of sickle cell anemia. Second, they are rapidly performed, the total time frequently being appreciably less than the time required to perform the routine sedimentation rate. Third, they require only simple laboratory equipment. Fourth, they offer themselves as additional objective tests for use in the study of doubtful cases.

**Methods.** These studies are based on observations made on 26 patients with sickle cell anemia and 60 who were normal subjects or had other diseases. The diseased states included luetic hepatitis, peptic ulcer, aplastic anemia, essential, benign and malignant hypertension, pernicious anemia, malnutrition, amebiasis, carcinoma of the stomach, carcinoma of the esophagus, carcinoma of the rectum, pregnancy, acute and chronic gastritis, luetic heart disease, gout, ulcerative colitis, pulmonary tuberculosis, pyelitis, tuberculous peritonitis, disseminated lupus erythematosus, pneumococcus and virus pneumonia, Weil's disease, myelogenous leukemia and acromegaly. A total of 371 determinations were made on the blood of patients with sickle cell anemia and 239 determinations on the control patients. These observations indicated the characteristic effects of  $O_2$  and  $CO_2$  upon the sedimentation rate of the erythrocytes of sickle cell anemia and led to the formulation of two diagnostic procedures which were studied in detail in 14 patients with sickle cell anemia and 20 with other disorders. The two suggested diagnostic procedures are as follows:

1. *Oxygen-Carbon Dioxide Technique.* A tourniquet is placed on the arm and blood is removed. The entire operation should not take more than 60 seconds. This is important to keep the collection standard. Approximately  $9\frac{1}{2}$  cc. of venous blood are placed in a 10 cc. vial containing 6 mg. of dry ammonium oxylate and 4 mg. of dry potassium oxylate. Five cc. is sufficient, however. In these studies the larger volume was used in order that duplicates could be made. The vial is immediately corked, and rotated for not longer than 3 minutes. Half of the blood is poured into a flask containing oxygen and half into one containing carbon dioxide.\* These are rotated intermittently for 15 minutes after which sedimentation rates are determined for each blood sample according to the method described by Wintrobe.<sup>4</sup>

2. *Aeration-Tourniquet Technique.* Venous blood is removed under standard conditions as described above. Five cc. of blood is placed in a small beaker or Erlenmeyer flask and is rotated in air for 15 minutes after which a sedimentation rate is determined. A sphygmomanometer cuff inflated to a pressure just above the diastolic is allowed to remain on the arm for 6 minutes after which blood is drawn and a second sedimentation rate is determined.

It has been found (these studies and previous ones<sup>2</sup>) that the method functions as well and may be simplified if the tourniquet (inflated blood pressure

\* Oxygen and carbon dioxide had previously been run into 2 separate 50-cc. Erlenmeyer flasks. These were corked with rubber stoppers and were stored until ready for use. The amount of gas lost while pipeting the blood from the vial to the flask did not alter the results, as 100% gas is not necessary for the test (*vide infra*).



cuff) is applied for 6 minutes and 5 cc. of blood is collected as described above. A sedimentation rate is then determined immediately on the blood; the remainder is rotated for 15 minutes in air in a small Erlenmeyer flask and then a sedimentation rate is determined on the aerated blood. This procedure offers a definite advantage in that a venepuncture is performed only once.

**Results.** The results of the oxygen-carbon dioxide test are summarized in Table 1. It may be seen that in patients with sickle cell anemia, oxygen accelerates and carbon dioxide retards the sedimentation rate compared with that of venous blood run immediately upon removal from the patient. There was a mean acceleration by  $O_2$  of 28.3 mm. of the initial sedimentation rate of venous blood (the rate being doubled) and a mean deceleration by  $CO_2$  of 23.3 mm. (the rate being reduced more than 20 times).

TABLE 1.—THE EFFECT OF  $O_2$  AND  $CO_2$  ON THE SEDIMENTATION RATE OF 8 PATIENTS WITH SICKLE CELL ANEMIA AND 14 PATIENTS WITH OTHER CONDITIONS

(The "diagnostic parameters" [ $\Delta$ ] are listed in the last column)

Subject No., or diagnosis	No. of individual determina- tions	Average sedimentation rate (Wintrobe) (mm./hr.)			Acceleration of SR. (SR. in O <sub>2</sub> ) minus (SR. in ven- ous blood) (mm./hr.)	Retardation of SR. (SR. in ven- ous blood) minus (SR. in CO <sub>2</sub> ) (mm./hr.)	Diagnostic parameter (SR. in O <sub>2</sub> ) minus (SR. in CO <sub>2</sub> ) (mm./hr.)
		Venous blood	O <sub>2</sub>	CO <sub>2</sub>			
<i>Sickle Cell Anemia Patients</i>							
1	4	36.0	40.0	0.0	4.0		40.0
2	1	35.0	70.0	0.0	35.0	35.0	70.0
3	2	14.0	62.0	1.0	48.0	13.0	61.0
4	1	7.0	67.0	3.0	60.0	4.0	64.0
5	2	31.0	51.0	1.0	20.0	30.0	50.0
6	2	11.0	40.0	1.0	29.0	10.0	39.0
7	3	26.0	52.0	2.0	26.0	24.0	50.0
8	1	36.0	41.0	1.0	5.0	35.0	40.0
Mean	...	24.5	52.8	1.1	28.3	23.3	51.7
Maximum	...	36.0	70.0	3.0	60.0	36.0	70.0
Minimum	...	7.0	40.0	0.0	4.0	4.0	39.0
<i>Control Patients</i>							
Pernicious anemia	4	70.0	66.0	60.0	-4.0	10.0	6.0
Peptic ulcer	1	16.0	20.0	13.0	4.0	3.0	7.0
Peptic ulcer	1	60.0	59.0	56.0	-1.0	4.0	3.0
Peptic ulcer	1	66.0	65.0	63.0	-1.0	3.0	2.0
Amebiasis	1	48.0	46.0	40.0	-2.0	8.0	6.0
Ca. stomach	1	50.0	50.0	41.0	0.0	9.0	9.0
Malnutrition	1	50.0	48.0	31.0	-2.0	19.0	17.0
Pernicious anemia	1	35.0	39.0	20.0	4.0	15.0	19.0
Pregnancy	1	60.0	61.0	60.0	1.0	0.0	1.0
Malnutrition	1	34.0	31.0	23.0	-3.0	11.0	8.0
Ca. rectum	1	15.0	20.0	15.0	5.0	0.0	5.0
Gastritis	1	32.0	38.0	24.0	6.0	8.0	14.0
Cardiac disease	1	55.0	55.0	47.0	0.0	8.0	8.0
Normal patient	1	6.0	5.0	4.0	-1.0	2.0	1.0
Mean	...	42.6	43.1	35.5	0.4	7.1	7.5
Maximum	...	70.0	66.0	63.0	6.0	19.0	19.0
Minimum	...	6.0	5.0	4.0	-4.0	0.0	1.0

The relative effect of saturating the blood of patients with sickle cell anemia with oxygen, or carbon dioxide, on the sedimentation rate, depends on the initial sedimentation rate of the venous blood. If the initial sedimentation rate of venous blood is slow, the oxygen will pro-

duce a marked acceleration of rate, whereas the carbon dioxide will produce a slight retardation. Conversely, when the initial sedimentation rate of venous blood is rapid, the acceleration of the rate by oxygen is slight and the retardation by carbon dioxide is marked.

The results of the aëration-tourniquet test are summarized in Table 2. In patients with sickle cell anemia, aëration of venous blood accelerates and the application of a tourniquet retards the sedimentation rates when compared with the sedimentation rate of unaltered venous blood.

TABLE 2.—THE EFFECT OF AÉRATION OF BLOOD AND THE APPLICATION OF A TOURNIQUET ON THE SEDIMENTATION RATE IN 6 PATIENTS WITH SICKLE CELL ANEMIA

(The "diagnostic parameter"  $[\Delta_2]$  has been calculated as indicated in the heading of the last column)

Subject No. or diagnosis	No. of individual determina- tions	Average sedimentation rate (Wintrobe) (mm./ hr.)			Acceleration of SR. (SR. after aëration) minus (SR. in ven- ous blood) (mm./hr.)	Retardation of SR. (SR. in ven- ous blood) minus (SR. after tourniquet) (mm./hr.)	Diagnostic parameter (SR. after aëration) minus (SR. after tourniquet) (mm./hr.)
		Ven- ous blood	After aëra- tion	After tourni- quet			
<i>Sickle Cell Anemia Patients</i>							
1	1	7.0	69.0	3.0	62.0	4.0	66.0
2	2	6.0	39.0	2.0	33.0	4.0	37.0
3	1	1.0	66.0	1.0	65.0	0.0	65.0
4	1	7.0	39.0	1.0	32.0	6.0	38.0
5	1	31.0	68.0	3.0	37.0	28.0	65.0
6	2	36.0	40.0	3.0	4.0	33.0	37.0
Mean	..	14.6	53.5	2.1	38.8	12.5	51.3
Maximum	..	36.0	69.0	3.0	65.0	33.0	66.0
Minimum	..	1.0	39.0	1.0	4.0	0.0	37.0
<i>Control Patients</i>							
Luetic hepatitis	1	16.0	16.0	11.0	0.0	5.0	5.0
Peptic ulcer	1	47.0	46.0	49.0	-1.0	-2.0	-3.0
Aplastic anemia	1	50.0	50.0	48.0	0.0	2.0	2.0
Essential hypertension	1	49.0	48.0	47.0	-1.0	2.0	1.0
Pernicious anemia	1	30.0	30.0	26.0	0.0	4.0	4.0
Malnutrition	1	26.0	25.0	20.0	-1.0	6.0	5.0
Mean	..	36.3	35.8	33.5	-0.5	2.8	2.3
Maximum	..	49.0	50.0	47.0	0.0	6.0	5.0
Minimum	..	16.0	16.0	11.0	-1.0	-2.0	-3.0

Aëration of venous blood produced a mean increase in the sedimentation rate of 38.8 mm. over that for venous blood (an increase of about 2.5 times), while venous stasis produced by a tourniquet resulted in a mean decrease in the sedimentation rate of 12.5 mm. (a decrease of 6 times). Here, as in the oxygen-carbon dioxide method, the degree of acceleration, or retardation depends upon the initial sedimentation rate of venous blood. It is to be noted that the average acceleration of the sedimentation rate due to aëration is greater than the retardation due to the application of the tourniquet (Table 4).

The effects produced by the two procedures, upon the sedimentation rates of the erythrocytes of sickle cell anemia is considerably greater than that for the control subjects (Tables 1 and 2).

**The Diagnostic Parameter.** In order to utilize the lability of the sedimentation rate so that it might aid in the diagnosis of sickle cell

TABLE 3.—STATISTICAL ANALYSIS OF DATA PRESENTED IN TABLE 1

	(SR. in O <sub>2</sub> ) minus (SR. in venous blood) (mm./hr.)	(SR. in venous blood minus (SR. in CO <sub>2</sub> ) (mm./hr.)	Diagnostic parameter (SR. in O <sub>2</sub> ) minus (SR. in CO <sub>2</sub> ) (mm./hr.)
<i>Sickle Cell Anemia Patients</i>			
Mean	28.3	23.3	51.7
Estimated standard deviation	±19.4	±12.7	±12.0
Standard error of mean	±6.8	±4.5	±4.2
Mean			
Standard error of mean	4.1	5.2	12.2
Probability	<0.01	<0.01	<0.01
<i>Control Patients</i>			
Mean	0.4	7.1	7.5
Estimated standard deviation	±3.1	±5.6	±5.6
Standard error of mean	±0.8	±1.5	±1.5
Mean			
Standard error of mean	0.5	4.8	5.0
Probability	>0.6	<0.01	<0.01
<i>Comparison of Means</i>			
Difference	27.9	16.2	44.2
Standard error of difference	±5.2	±3.9	±3.8
Difference			
Standard error of difference	5.4	4.2	11.6
Probability	<0.01	<0.01	<0.01

TABLE 4.—STATISTICAL ANALYSIS OF DATA PRESENTED IN TABLE 2

	(SR. after aëration) minus (SR. in venous blood) (mm./hr.)	(SR. in venous blood) minus (SR. after tourniquet) (mm./hr.)	Diagnostic parameter (SR. after aëration) minus (SR. after tourniquet) (mm./hr.)
<i>Sickle Cell Anemia Patients</i>			
Mean	38.8	12.5	51.3
Estimated standard deviation	±22.4	±14.2	±15.3
Standard error of mean	±9.2	±5.8	±6.2
Mean			
Standard error of mean	4.2	2.2	8.2
Probability	<0.01	>0.05	<0.01
<i>Control Patients</i>			
Mean	-0.5	2.8	2.3
Estimated standard deviation	±0.5	±2.8	±3.07
Standard error of mean	±0.2	±1.2	±1.2
Mean			
Standard error of mean	2.2	2.4	1.8
Probability	>0.05	>0.05	>0.01
<i>Comparison of Means</i>			
Difference	39.3	9.7	49.0
Standard error of difference	±9.2	±5.9	±6.4
Difference			
Standard error of difference	4.3	1.6	7.7
Probability	<0.01	>0.1	<0.01

anemia, the degree of acceleration and retardation by  $O_2$  and  $CO_2$  respectively, must be considered in relation to each other. In order to do this, a "diagnostic parameter" is calculated (Tables 1 and 2), as described below.

1. When the oxygen-carbon dioxide technique is used, the diagnostic parameter ( $\Delta_1$ ) may be stated as the difference between the sedimentation rate in oxygen and the sedimentation rate in carbon dioxide:  $\Delta_1 = \psi_1$ .  $\psi_1$  is equal to the sedimentation rate in  $O_2$  minus the sedimentation rate in  $CO_2$ . If  $\Delta_1$  is greater than 27 mm. per hour, the test is "positive" and it is probable (98 chances out of 100) that the patient has active sickle cell anemia (Fig. 1). The value 27 was determined statistically (see Appendix).

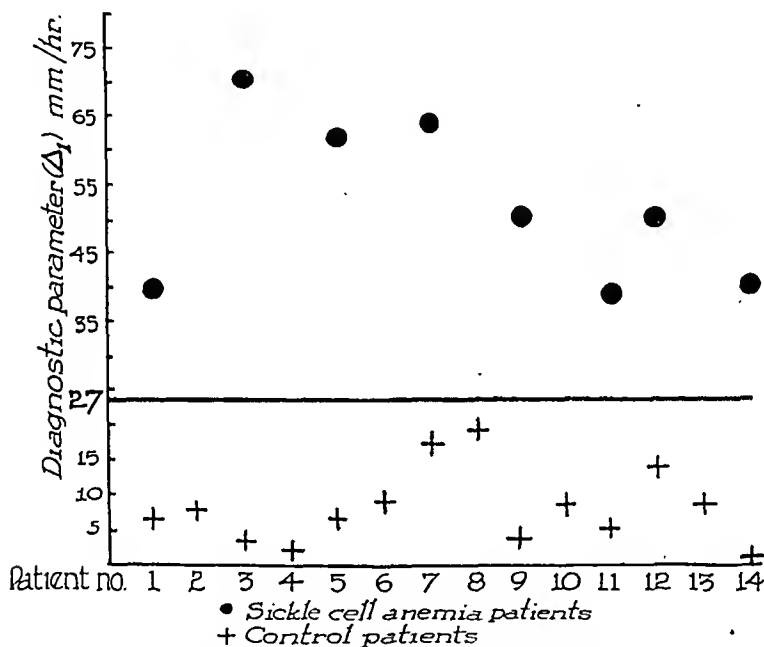


FIG. 1.—The diagnostic parameters ( $\Delta_1$ ) obtained by means of the oxygen-carbon dioxide technique in 8 patients with sickle cell anemia and 14 patients with other conditions. The values are shown in relation to the parameter of 27 mm./hr., a value found statistically, which separates patients with sickle cell anemia from those with other clinical states.

2. When the aëration-tourniquet technique is used, the diagnostic parameter ( $\Delta_2$ ) may be stated as the difference between the sedimentation rate of the aërated blood and the sedimentation of the non-aërated blood after the application of a tourniquet:  $\Delta_2 = \psi_2$ .  $\psi_2$  is equal to the sedimentation rate after aëration minus the sedimentation rate of non-aërated blood after applying the tourniquet. If the  $\Delta_2$  is greater than 20 mm. per hour, the test is "positive" and it is probable (98 chances out of 100) that the patient has active sickle cell anemia (Fig. 2). The value 20 was determined statistically (see Appendix).

**Discussion.** The erythrocytes of patients with sickle cell anemia are peculiar in their response to saturation with  $O_2$  and  $CO_2$ . The for-

mer produces a marked acceleration of the sedimentation rate, while the latter produces a marked retardation. These effects may be found to a slight degree in patients with other clinical states, but not nearly to the extent seen in sickle cell anemia. The consistency and the range of these changes immediately suggested their diagnostic, as well as their physicochemical importance. In fact, when the acceleration and retardation of the sedimentation rate by these two gases are expressed as functions of each other in order to arrive at a value that might have diagnostic significance and at the same time control the variability of the sedimentation rate as found in venous blood, a significant expression was obtained. This expression was arbitrarily

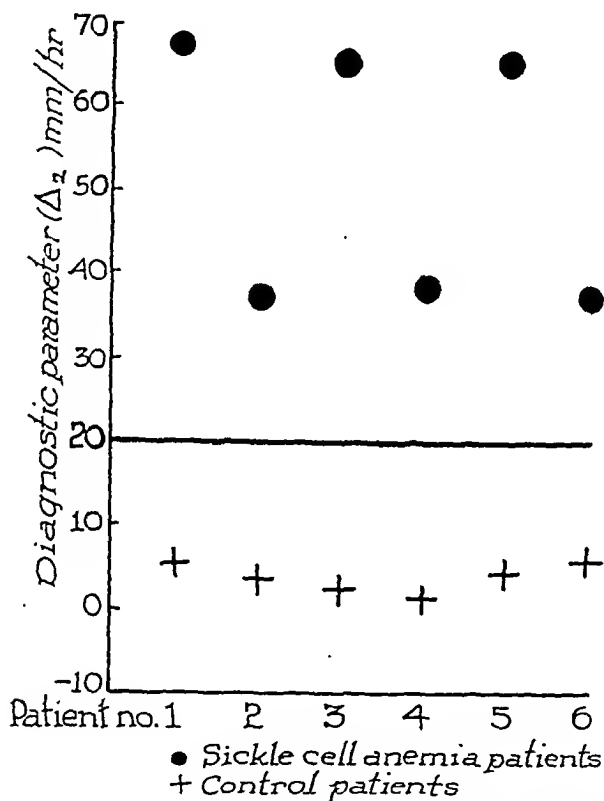


FIG. 2.—The diagnostic parameters ( $\Delta_2$ ) obtained by means of the aëration-tourniquet technique in 6 patients with sickle cell anemia and 6 patients with other conditions. The values are shown in relation to the parameter of 20 mm./hr., a value found statistically, which separates patients with sickle cell anemia from those with other clinical states.

called the "diagnostic parameter." When these data were tested statistically, it was found to have a 98% accuracy in the diagnosis of sickle cell anemia. The control subjects had 4 chances in much more than 10,000 of falling within the range for sickle cell anemia blood when the oxygen-carbon dioxide test is used, and 1 chance in much more than 10,000 of falling within the range of sickle cell anemia blood when the aëration-tourniquet test is used (see Appendix for statistical data).

The aëration-tourniquet test is the more simple of the two procedures

since it does not require that pure  $O_2$  and  $CO_2$  be available. All that is needed are sedimentation tubes, a clinical blood pressure apparatus and syringe and needle. Furthermore, this method can be simplified so that only 1 venepuncture is necessary (see Method).

It is not suggested that these tests be used to replace the microscopic study of the erythrocyte for the sickling phenomenon. They are to be used when one is in need of quick diagnosis, as would be encountered when the abdominal crises are confused with acute surgical conditions in the abdomen. It is not the purpose of this discussion to point out the dangers of surgical procedures in these patients.

It has been found in most instances, that after the blood has been placed in the sedimentation tubes, the diagnostic parameter is reached within 15 to 20 minutes. It is not necessary to carry the test out to a full hour under those circumstances, thus reducing the entire time for the test to about 30 minutes.

**Summary.** The marked retarding of the sedimentation rate of the erythrocytes of patients with sickle cell anemia by  $CO_2$  and the marked acceleration by oxygen were used as a basis for two simple rapid macroscopic tests for active sickle cell anemia. These tests were found to be 98% reliable in the identification of sickle cell anemia (see Appendix).

The aëration tourniquet test is the simpler to perform and requires less material. The method can be simplified to involve the following procedures:

1. A tourniquet (blood pressure cuff) is applied to an arm to produce venous stasis for 6 minutes.
2. Five cc. of blood are drawn from a vein and placed in a small vial containing an anticoagulant, stoppered and rotated gently in order to avoid mixing the blood with air.
3. A sample of the blood is set up for a sedimentation measurement immediately.
4. The remainder of the blood is rotated in air in a small beaker, or Erlenmeyer flask, to saturate it thoroughly. A sedimentation rate is then determined on this aërated blood.
5. Within 15 to 60 minutes the diagnostic parameter ( $\Delta_2$ ) should be greater than 20 mm. per hour if the patient has sickle cell anemia. As soon as this parameter is reached, it is not necessary to continue the sedimentation rate to a full hour.

**Conclusion.** The aëration-tourniquet test not only is simple and rapid, but it serves as a check on the microscopic study of sickling which is often not properly done, or is late in showing evidence of sickling—often too late for the proper management of diagnostic problems of acute abdominal conditions which may, or may not, require surgical intervention.

## APPENDIX

### STATISTICAL ANALYSIS

*Oxygen-Carbon Dioxide Technique.* The diagnostic parameter ( $\Delta_1$ ) shown in Table 1 is the difference between the sedimentation rate in  $O_2$  and the sedi-

mentation rate in  $\text{CO}_2$  and thus is independent of the sedimentation rate in venous blood. The mean values (Table 3) for  $\Delta_1$  for both the sickle cell anemia patients and the control patients are statistically reliable. The probability of less than 0.01 ( $P < 0.01$ ) means that in less than 1% of such samples of sickle cell anemia patients and control patients are mean values, as great or greater than those shown in Table 1, likely to be obtained by chance alone.

The difference between the mean values of the diagnostic parameter for these two groups of patients is sufficient statistical evidence that there are factors present in the sickle cell anemia patients producing a high diagnostic parameter which are evidently absent in the control patients.

*Aëration-Tourniquet Technique.* The diagnostic parameter ( $\Delta_2$ ) shown in Table 2 is the difference between the sedimentation rate after aëration and the sedimentation rate after application of the tourniquet. The mean value shown in Table 4 ( $\Delta_2$ ) for the sickle cell anemia patients on whom this technique was used is statistically reliable, the probability being determined as  $P < 0.01$ . The mean value of the diagnostic parameter for the control patients in whom this technique was used is not statistically reliable, because there is no statistical evidence of consistent alteration of the sedimentation rate produced by either the application of the tourniquet, or the aëration of the blood.

The analysis stated above for the difference between the mean values of the diagnostic parameter for the two groups of patients on whom the  $\text{CO}_2\text{-O}_2$  technique was used also applied when using the tourniquet-aëration method.

*Lower Limit of the Diagnostic Parameter.* A value of 27 mm./hr. is established as the lower limit of the diagnostic parameter ( $\Delta_1$ ) accepted for the diagnosis of sickle cell anemia using the  $\text{O}_2\text{-CO}_2$  technique. This value equals the computed mean of  $\Delta_1$  for the sickle cell anemia patients minus 2.05 estimated standard deviations ( $M - 2.05 \sigma$ ). Approximately 98% (97.98%) of sickle cell anemia patients may be expected to have a diagnostic parameter equal to or greater than this value. Assuming that the diagnostic parameter obtained for the control patients represents those values that would be obtained for patients with other types of anemia and infections, the probability that any such patient would have a diagnostic parameter greater than 27 mm. hr. is determined as  $P < 0.0004$ . Thus less than 4 of 10,000 patients are likely to be incorrectly diagnosed as having sickle cell anemia.

A value of 20 mm./hr. was established as the lower limit of the diagnostic parameter ( $\Delta_2$ ) accepted for the diagnosis of sickle cell anemia using the aëration-tourniquet technique. This value equals the computed mean of  $\Delta_2$  for the sickle cell anemia patients minus 2.04 estimated standard deviations ( $M - 2.04 \sigma$ ). Approximately 98% (97.93%) of sickle cell anemia patients may be expected to have a diagnostic parameter equal to or greater than this value. Again assuming that the diagnostic parameter obtained for the control patients represents those values that would be obtained for patients with other types of anemia and infections, the probability that any such patient would have a diagnostic parameter greater than 20 mm./hr. is determined as  $P < 0.0001$ . Thus less than 1 of 10,000 patients on whom the aëration-tourniquet technique is used are likely to be incorrectly diagnosed as having sickle cell anemia.

We wish to express our appreciation to Mr. R. J. Hammerstrom, of the Department of Preventive Medicine, for his valuable help in these statistical studies.

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HAND-SCHÜLLER-CHRISTIAN'S SYNDROME AND "EOSINOPHILIC  
OR SOLITARY GRANULOMA OF BONE"

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SNAPPER,<sup>7</sup> in a chapter devoted to Schüller-Christian's disease refers to patients suffering from lipoidal granulomas of the long bones with absence of granulomas of the cranium. He also mentions the hypothesis that the proliferation of the reticulo-endothelial system with granuloma formation is a primary phenomenon of these xanthomatoses, and insists particularly on the importance of choosing the lesion from which the biopsy is to be made in order to avoid mistakes in diagnosis.

The publications of Otani and Ehrlich,<sup>6</sup> Lichtenstein and Jaffe,<sup>5</sup> Bass,<sup>1</sup> Farber,<sup>2</sup> Green, and Farber<sup>3</sup> and Gross and Jacox<sup>4</sup> produced more extensive discussion of this subject; clinicians and pathologists are still attempting to clear up divergencies as to the nature of Hand-Christian's disease and of eosinophilic or solitary granuloma of the bones, isolated xanthomas and the non-lipoid reticulo-endotheliosis of Letterer-Siwe.

We consequently, in this paper, offer this contribution towards elucidating the question.

**Report of a Case.** The patient, M. Q. (Fig. 1), Spanish, 50 years old, resident in Brazil since she was 40 days old, married at 16, and has had 15 pregnancies, of which 12 children are alive and physically well developed; 1 child died suddenly when it was 27 days old, cause unknown. The first and last pregnancies ended in abortion at 4 months. After the last abortion, in the middle of 1940, there followed a period of menorrhagia which lasted 8 months and was cured by uterine curettage. She has suffered from measles but there is no history of traumatism.

While suffering from menorrhagia she began to feel, in the central region of the left thigh, stabbing pains followed by short periods of relief. The Roentgen ray examination made in Rio de Janeiro in April, 1941, showed a fusiform area of rarefied bone in the middle of the diaphysis of the left femur (Fig. 2). The Wassermann and Kahn tests were negative, but Müller's test was slightly positive, but negative on August 9, 1941, after antisyphilitic treatment. The patient's painful symptoms were relieved and she returned to her home in Minas. The teeth, which by then were loose, began to fall out and shortly afterwards the pain the left thigh reappeared, now however, radiating to the hip and knee on the same side. On May 20, 1942, while hurrying to catch a train, she suffered a fracture of the left thigh (Fig. 3) and was admitted to First-Aid Hospital.





FIG. 1.—Patient M. Q.; no exophthalmia can be seen.



FIG. 2.—Radiograph of the middle part of the diaphysis of the left femur showing the shadow of the rarefied lacuna in April, 1941.

*Physical Examination.* The patient was badly nourished. Many teeth were lost, the few remaining being secured only by the soft tissues. There was pyorrhea and polydipsia; no exophthalmia; the sclera slightly bluish; the fundi normal. There was a pathologic fracture at the junction of the middle and upper third of the left femur occurring near an oval lacuna shadow of irregular outline measuring about 6 cm. long. Below this there were 2 other smaller shadows of identical aspect. The compact bone tissue was attacked (Fig. 3). The liver and spleen were normal.



FIG. 3.—Radiograph of the middle part of the diaphysis of the left femur showing fracture at the set of osseous rarefaction in May, 1942.

*Laboratory Examinations.* Urine: volume in 24 hours, 5780 cc.; density at 150, 1006; no albumin, glucose or other abnormal substances (including Bence-Jones' protein). Blood: hemoglobin, 9.92 gm. (Sahli); erythrocytes, 4,065,000; leukocytes, 8400 (neutrophils, 48%; eosinophils, 6.5%; mast cells, 1%; lymphocytes, 40%; monocytes, 4.5%). Glucose, 74 mg. per 100 cc.; uric acid, 2.48 mg. per 100 cc.; cholesterol, 134; inorganic phosphates, 2.8; calcium, 13.5; phosphatase (Kay), 0.08 unit. In the feces there were eggs of *A. lumbricoides* and *T. trichiura*.

Myelogram (sternal puncture): Hemohistoblasts with neutrophil granules, 0.4%; proneutrophil myeloblasts, 0.4%; neutrophil promyelocytes, 2.6%; neutrophil myelocytes, 13.6%; neutrophil metamyelocytes, 8.2% polymorphonuclear neutrophils, 36%; eosinophil myelocytes, 2.2%; eosinophil metamyelocytes, 1.8%; polymorphonuclear eosinophils, 3.2%; lymphocytes, 10.4%; mast cells, 0.2%; monocytes, 0.2%; megaloblasts, 0.4%; megaloblasts in mitosis, 0.2%; proerythroblasts, 0.4%; basophil erythroblasts, 1.4%; polychromatophil erythroblasts, 0.6%; orthochromatic erythroblasts, 15%; megacaryocytes, 0.2%; plasma cells, 2.4%; Türk cells, 0.2%.

Roentgen ray films of skull bones, pelvis, right femur, tibia, humerus, ribs, maxillary bones and lungs showed nothing of interest.

*Diagnosis.* Intestinal worms. Pathologic fracture of the left femur. Hand-Christian's syndrome.

The curetting of the focus of fracture, as the first stage towards irradiation or bone grafting, was done by one of us (J. M. F.) on May 5, and showed the existence of a yellowish, spongy-looking tissue projecting from the fractured part and extending along the medullary zone of the femur; samples of this were taken for histopathologic examination.

*Pathologic Report* (M. A. J.). No. 3857. Ten small samples, previously fixed in alcohol, were sent for examination; all the material was embedded in paraffin.



FIG. 4.—Eosinophilic infiltration of the granuloma.

The sections showed that large areas of these fragments had undergone necrosis with hemorrhage, and around these areas could be seen rounded cells with abundant protoplasm enclosing, in phagocytic fashion, a greenish-yellow pigment (a hematic pigment giving the Berlin blue reaction). Among these cells there were some multinuclear ones which at times also contained pigment. In the peripheral zone of some samples, the reticulo-endothelial structure could be identified with thickening of the reticulum and cellular hyperplasia, here also were some multinuclear cells, looking like Reed-Sternberg cells.

Some areas were infiltrated by eosinophils which, in some places, were so compact that they produced the appearance of a true eosinophil granuloma (Fig. 4).

One sample looked like xanthomatous tissue, consisting of bulky cells with central nucleus and foam-like protoplasm; some multinuclear giant cells (Touton cells) were also seen (Figs. 5 and 6). All these cells lay amidst a thickened reticulum, and in a limited peripheral area, some mast cells could be seen. In this sample only 2 small trabeculae were seen, one of them with signs of decalcification.

*Treatment and Evolution.* The treatment prescribed by us was vitamins A and D and extract of the posterior lobe of the hypophysis. The latter was at first administered through the skin (Retrophysina) and later through the nose (retrohypophysis powder—Slopart's snuff). There was no marked altera-

tion of blood pressure. The polydipsia disappeared and the flow of urine became normal; but both these conditions have, up to the present, reappeared when the hypophysis extract medication is discontinued. As a proof of this it is sufficient to state that on January 11, 1943, after a 3 day interruption of the hypophysis medication, the volume of urine increased to 12 liters in 24 hours, and the calciuria was 380 mg. (expressed as calcium—Shohl and Pedley's method). On the same date the serum's calcium content was 12.5 mg. per 100 cc., the phosphates 4.8 mg. per 100 cc. and the phosphatase 0.19 unit (Kay).



FIG. 5.—Giant cells (Touton-like) and foam cells.

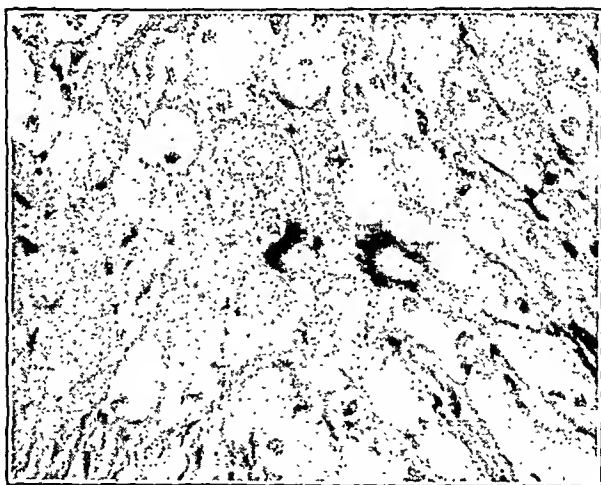


FIG. 6.—Giant cells (Touton-like) and foam cells.

A new Roentgen ray examination at the end of December, 1942, showed that the bony callosity was being slowly but aberrantly formed, giving, for the time being, an impression of fragility.

In no other part of the bony skeleton was any lacuna of rarefaction observed, the left tibia, however, showed greater permeability to the Roentgen rays, apparently a process of decalcification. We have tried to readjust the amounts of vitamins A and D as well as the supply of calcium in the food and the patient continues under observation.

**Comment.** Even if we restrict the use of the name Hand-Christian's disease to the morbid condition in which the classical triad of symptoms are present, *viz.* (1) multiple lacuna shadows in the cranial bones, (2) exophthalmia and (3) diabetes insipidus, the diagnosis of Hand-Christian's syndrome can be justified in our case by virtue of the presence of a rarefying osseous lesion of the left femur and diabetes insipidus, because this latter symptom leads us to suspect a lesion of the hypophysis although it has not yet been possible to demonstrate this radiologically.

The histologic examination of the material taken from the seat of fracture showed the existence of a granulomatous lesion which in a certain zone showed accumulation of eosinophils giving the picture of eosinophil granuloma; in another zone there were lipophages (foam cells), arranged like a typical lipogranuloma.

The cellular hyperplasia and thickening of the reticulum were apparent in the preparations.

We thus arrive at the following conclusion:

**Conclusion.** In accord with Farber, Bass, Gross and Jacox, and based on our own observations, we are of the opinion that the clinical pictures referred to in this paper should be regarded as variations of the same morbid process, which in a more advanced stage is known as Hand-Christian's disease.

We are grateful for the invaluable collaboration of Prof. Oromar Morcira, Dr. A. C. Cavaleanti, Dr. Edgard A. Cerqueira and Dr. Paulo Rocha, who kindly carried out the chemical dosage and radiologic examinations, and to Prof. Ollivierre who made the translation into English.

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### SULFONAMIDE INHIBITING ACTION OF PROCAINE

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THE original method of Marshall<sup>16</sup> and its later modifications<sup>2,6</sup> for determining sulfanilamide depend on diazotization and the coupling of the resulting diazo compound to produce an azo dye which can be

easily estimated by colorimetric comparison. The reaction depends on the presence of an amino group substituted in the benzene ring and can, therefore, be used for the estimation of any derivative of sulfanilamide or for any related aromatic compound in which the amino group is free or can be freed by hydrolysis. Recognition of this fact has served to explain inconsistencies in the determination of sulfonamide drugs in body fluids.<sup>3</sup> Procaine ("novocaine"), which is the local anesthetic most commonly employed in obtaining such body fluids, is  $\beta$ -diethylaminoethyl-p-aminobenzoate<sup>11</sup> and thus has a free aryl amine which may enter this reaction. The procaine may contaminate the test fluid either from its presence in the needle and syringe or by admixture with the fluid withdrawn after procaine infiltration.<sup>1,4,17</sup> Failure to recognize this possibility is probably responsible for gross misinterpretations and erroneous results.<sup>10,12</sup> Other local anesthetics not derived from p-aminobenzoic acid do not have this interfering effect.<sup>11,13,17</sup>

Woods<sup>23</sup> was the first to demonstrate that p-aminobenzoic acid and related compounds, including procaine, exert a marked inhibiting effect on the bacteriostatic action of sulfanilamide on hemolytic streptococci *in vitro*. The inhibiting effect of procaine was found to be quantitatively similar to that of p-aminobenzoic acid but it was somewhat delayed, probably because the compound was hydrolyzed by the organisms. Sulfapyridine required 5 times as much p-aminobenzoic acid as was needed for sulfanilamide to produce the same degree of inhibition. This was interpreted as indicating that sulfapyridine was 5 times as active as sulfanilamide. Selbie,<sup>19</sup> at the same time, showed that this inhibiting action of p-aminobenzoic acid can also be demonstrated *in vivo* in mice infected with hemolytic streptococci and treated with sulfanilamide. Boroff, Cooper and Bullowa<sup>1</sup> estimated the average concentration of procaine in pleural fluid removed after the use of this agent to be 0.0002% (0.2 mg. per 100 cc.) and they showed that this amount was sufficient to inhibit *in vitro* the action of at least 0.005% (5 mg. per 100 cc.) of sulfapyridine on Type III pneumococci. Larger concentrations of urethane, which is not a p-aminobenzoic acid derivative, did not exert this inhibiting effect. Similar observations on the inhibiting action of p-aminobenzoic acid compounds have been made both *in vitro* and *in vivo* with the same or other sulfonamides and with other organisms, including *E. coli*, *Staphylococcus aureus* and Type I pneumococcus.<sup>13,15</sup>

Casten, Fried and Hallman<sup>5</sup> have shown that in rabbits procaine inhibits the bacteriostatic action of sulfathiazole in wounds inoculated at the time of trauma with suspensions of *Staphylococcus aureus*. They concluded from their observations that procaine is contraindicated as a local anesthetic in the treatment of open wounds or compound fractures if sulfathiazole is to be used topically. DeWaal, Kanaar and McNaughtan<sup>7</sup> using hemolytic streptococcus infections in mice found that massive non-toxic doses of procaine given at short intervals were necessary in order to produce any significant antisulfanilamide action. They also cited 3 cases of human hemolytic strepto-

coccal and pneumococcal infections in which sulfapyridine was given orally and no discernible antisulfonamide action resulted from the local injections of procaine in amounts varying from 60 cc. of a 1% solution to 50 cc. of a 3% solution (0.6 to 1.5 gm.). The procaine was used, at least 24 hours after the sulfapyridine was started—for incision of infected wounds in 1 of these cases and to infiltrate the pleura for the relief of pain in the other 2 cases.

Legge and Durie<sup>14</sup> presented evidence that procaine is hydrolyzed to p-aminobenzoic acid by an esterase present in human blood and in mice. Among mice injected with hemolytic streptococci and treated with sulfanilamide, the injection of procaine caused a slight increase in the mortality rate. Although they presented no clinical data, they suggested that procaine and other local anesthetic agents of the p-aminobenzoic acid class are contraindicated when the patient is so seriously infected that even a slight delay in treatment with sulfonamides may diminish the chances of survival or when massive tissue damage is present and the circulation in the damaged tissue is poor so that the removal of p-aminobenzoic acid from the tissue after the use of procaine may be very slow.

Goldberg, Koster and Warshaw<sup>9</sup> showed that no appreciable concentration of procaine is built up in the blood during spinal anesthesia. Once it gets into the blood stream the procaine is rapidly hydrolyzed (detoxified) by an enzyme and some of the free amino group resulting from this hydrolysis is further acetylated by a less active enzyme and the products are rapidly excreted in the urine. An average of 90% of injected procaine is thus excreted in the urine but only in the form of the products of detoxification; these are more or less equally divided between p-acetaminobenzoic acid, p-acetaminohippuric acid and p-acetaminobenzoyl glycuronate.

In this laboratory<sup>21</sup> it has been shown that p-aminobenzoic acid given orally in humans is rapidly excreted in the urine, largely in conjugated form. This rapid excretion also occurs in mice and accounts for the necessity of using frequent and large doses of p-aminobenzoic acid to inhibit the action of sulfonamides, since the latter are excreted more slowly.<sup>7,22</sup>

The sulfonamide inhibiting effect of procaine thus seems well established insofar as it concerns bacterial growth in artificial media and, to a lesser extent, in experimental animals. In the latter, repeated injections of large doses of procaine seem to be essential when the sulfonamide is used to combat a general infection, but local infiltration with comparatively small doses of procaine seem to be sufficient to interfere with the action of sulfonamides applied topically for the prevention of wound infection. Thus far, little if any evidence has been brought forth to indicate that this sulfonamide inhibiting action of procaine is of significance in human infections. It is the purpose of this paper to present: 1, the results of studies which indicate that procaine as employed in local anesthesia, may inhibit the action of sulfonamides in human blood; and, 2, case reports illustrating the local sulfonamide-inhibiting action of procaine.

**Experimental. Materials and Methods.** The subjects for these studies were shown, in preliminary tests, to have little or no bactericidal action against the test organisms in their freshly shed defibrinated blood. Whenever possible, patients undergoing operations under local anesthesia with procaine were used in these studies. The stock strain of Type III pneumococcus was employed. The bactericidal tests with human blood were carried out as in previous studies.<sup>20</sup> Briefly, 0.5 cc. amounts of fresh defibrinated blood were placed in pyrex tubes; serial decimal dilutions of a fully grown culture were added in 0.1 cc. amounts and other ingredients were also added in a volume of 0.1 cc. The tubes were then sealed in a gas-oxygen flame and rotated slowly in an incubator at 37.5° C. The tubes were observed for growth as indicated by color change at 24 and 48 hours and by cultures of the blood in the tubes which failed to show a color change. When human serum was used, sterile glucose was added to a final dilution of 0.1% and growth was observed grossly or bacterial counts were done by making blood agar pour plates with dilutions of the resulting growth at the end of appropriate periods of incubation.

Chemical determinations of sulfonamide levels were made by the method of Bratton and Marshall<sup>2</sup> using a photoelectric colorimeter. The same method was used for the determination of procaine. When the blood contained both these chemicals, the total color change was read only with reference to the sulfonamide.

*Antisulfonamide Action of Procaine in Human Blood, in vitro.* It was shown in preliminary studies that the concentration of procaine obtained in the blood of patients undergoing local anesthesia in which a total of 0.5 to 2.5 gm. was used, ranged from 0.5 to 1.7 mg. per 100 cc. of the free procaine (namely, that portion circulating with a free aryl amine) with about an equal amount conjugated (probably acetylated on the amino group). Tests were, therefore, made *in vitro* with concentrations of procaine ranging from 0.1 to 1.5 mg. per 100 cc. and with amounts of sulfadiazine up to 20 mg. per 100 cc. The results of an experiment with Type III pneumococci in human blood are shown in Table 1. Procaine in concentrations of 1 mg. per 100 cc. or higher had a marked inhibiting effect in the bacteriostatic action of sulfadiazine in concentrations as high as 20 mg. per 100 cc. Smaller concentrations of the procaine had a moderate inhibiting effect on the action of smaller amounts of the sulfonamide.

TABLE 1.—EFFECT OF PROCAINE *in vitro* ON THE BACTERIOSTATIC ACTION OF SULFADIAZINE IN HUMAN BLOOD

Added to 0.5 cc. defibrinated blood			Added to 0.5 cc. defibrinated blood		
Sulfadiazine*	Procaine*	Bacteriostasis†	Sulfadiazine*	Procaine*	Bacteriostasis†
0	0	0	10	0.1	87,000
			10	0.5	870
2.5	0.1	87,000	10	1.0	0
2.5	0.5	87	10	1.5	0
2.5	1.0	0			
2.5	1.5	0	15	0.1	87,000
			15	0.5	870
5	0.1	87,000	15	1.0	0
5	0.5	87	15	1.5	0
5	1.0	0			
5	1.5	0	20	1.0	0
			20	1.5	0

\* Mg. per 100 cc.

† Largest number of Type III pneumococci which failed to show visible growth in 48 hours; in this column 0 = less than the smallest inoculum used (8 or 9 diplococci).



*Antisulfonamide Action of Procaine in Human Serum in vitro.* The results of one of a number of experiments are shown in Table 2. In this experiment sulfathiazole was used in concentrations up to 40 mg. per 100 cc. Here again, procaine in a concentration of 1 mg. per 100 cc. or higher permitted the free multiplication of Type III pneumococci in the presence of concentrations of sulfathiazole as high as 40 mg. per 100 cc. and 0.1 mg. of procaine per 100 cc. inhibited the action of 10 mg. per 100 cc. of sulfathiazole.

TABLE 2.—EFFECT OF PROCAINE *in vitro* ON THE BACTERIOSTATIC ACTION OF SULFATHIAZOLE IN HUMAN SERUM  
0.5 cc. serum + 6600 Type III pneumococci +

Sulfathiazole (mg./100 cc.)	Procaine (mg./100 cc.)				
	0	0.01	0.1	1.0	2.5
0	+	+	+	+	+
10	0	0	+	+	+
20	0	0	0	+	+
30	0	0	0	+	+
40	0	0	0	+	+

+ = heavy growth in 48 hours, 0 = no visible growth.

The following studies were carried out with blood obtained from human subjects in whom procaine was used either for local anesthesia in operative procedures or by subcutaneous or intramuscular injection given specifically for this purpose.

TABLE 3.—EFFECT OF PROCAINE *in vivo* ON THE BACTERIOSTATIC ACTION OF SULFADIAZINE IN HUMAN BLOOD

		Blood taken		
		Before sulfadiazine	Before procaine	After procaine
Subject A. Bacteriostasis*	Less than 8		8,200	8
	Sulfadiazine content†	0	F 7.3 T 8.4	F 7.7 T 8.4
Subject B. Bacteriostasis*	25		25,000	250
	Sulfadiazine content†	0	F 9.9 T 11.8	F 11.7 T 14.6
Subject C. Bacteriostasis*	4		44,000	44
	Sulfadiazine content†	0	F 8.1 T 10.9	F 7.9 T 11.6

\* Largest number of Type III pneumococci which failed to give visible growth in 48 hours.

† The sulfadiazine concentration (in mg. per 100 cc.). This includes procaine or its hydrolyzed product. On the basis of other observations the procaine should account for 0.5 to 1.7 mg. per 100 cc. (free). F = free. T = total.

A received 3 gm. of sodium sulfadiazine intravenously 3 hours before the procaine infiltration which took 30 minutes. The last blood was taken 40 minutes after a total of 2 gm. of procaine.

B received 2.5 gm. of sodium sulfadiazine intravenously 3 hours before procaine injection. A total of 2 gm. of procaine was injected subcutaneously in 30 minutes and blood was taken 1 hour later. (3 hours thereafter the blood level (calculated as sulfadiazine) was 9.5 mg. per 100 cc. free and 12.4 mg. per 100 cc. total, and 25,000 organisms were killed).

C received 3 gm. of sodium sulfadiazine intravenously 3½ hours before an intramuscular injection of 1 gm. of procaine in 30 cc. of saline and blood was obtained ½ hour later. After another 1½ hours the same number of organisms were killed and the blood level was 7.7 mg. per 100 cc. free and 11.6 mg. per 100 cc. total.

*Effect of Procaine, in vivo, on the Action of Sulfadiazine in Human Blood.* The results of studies in 3 patients are shown in Table 3.

Each of these subjects received an intravenous injection of sulfadiazine prior to the procaine injection. Blood was taken before each of the procedures and again at intervals after the injection of procaine. The greatest amount of inhibition was usually found to occur between  $\frac{1}{2}$  and 2 hours after the injection of procaine was completed. In each of these cases, although the exact amount of procaine in the blood was not determined, it is obvious that a marked inhibiting effect was exerted by the procaine on the antipneumococcal action of the sulfadiazine in the blood.

Similar experiments were carried out with sulfathiazole. With levels of this drug ranging from 2 to 4 mg. per 100 cc. injections of procaine totaling up to 1 gm. had only a slight inhibiting effect in some cases and none in others.

*Effect of Procaine in vivo in Human Serum.* A number of studies were made with serum obtained from patients who had received procaine injections. The effect of the procaine in the serum on the bacteriostatic action of added sulfathiazole was studied by bacterial counts made after varying periods of incubation. The result of one of these experiments is shown in Table 4. The concentration of "free" procaine was found to be 1.7 mg. per 100 cc. This amount of procaine had a definite inhibiting effect on the bacteriostatic action of sulfathiazole in concentrations as high as 25 mg. per 100 cc.

TABLE 4.—INHIBITING EFFECT OF PROCAINE *in vivo* ON THE BACTERIOSTATIC ACTION OF SULFATHIAZOLE ADDED TO HUMAN SERUM

Sulfathiazole added (mg./100 cc.)	Organisms per cc. after 48 hours growth in serum obtained	
	Before procaine	After procaine*
0 . . . . .	3,450,000,000	2,200,000,000
5 . . . . .	900,000,000	1,490,000,000
10 . . . . .	11,000,000	2,780,000,000
15 . . . . .	950,000	2,100,000,000
25 . . . . .	97,000	1,610,000,000

\* Inoculum, 8300 organisms per cc. of serum. Concentration of procaine per 100 cc. of serum: 1.7 mg. free, 3.3 mg. total. This serum was obtained from blood drawn  $\frac{1}{4}$  hour after subcutaneous injection of 1.5 gm. of procaine (without adrenalin).

*Cases Illustrating Local Inhibition of Sulfonamide Action by Procaine.* The results of the foregoing studies indicate that when procaine is used for local anesthesia in operative procedures (such as herniorrhaphies or operations on the gall bladder), or when equivalent amounts of this drug are injected subcutaneously or intramuscularly, concentrations of procaine or of derivatives containing a free aryl amine are absorbed into the blood in sufficient concentration to inhibit the bacteriostatic action of large concentrations of sulfadiazine or sulfathiazole. That procaine injections may permit the development of infection at the site of infiltration even while adequate concentrations of sulfonamides are maintained in the body, is illustrated in the following cases. Only the relevant details of these cases are presented.

**Case Studies.** CASE 1. This is one of the first cases of pneumococcal meningitis with recovery observed at the Boston City Hospital and many of the details have been reported elsewhere.<sup>8</sup> In this case, fever and symptoms of

meningitis appeared 2 weeks after the partial removal of a posterior fossa tumor in November, 1937. Sulfanilamide therapy was started at that time and continued over a period of about 9 weeks, a dose of 6 gm. daily being given during most of the first 6 weeks and 4 gm. daily thereafter. Blood levels of about 10 mg. per 100 cc. were maintained most of this time. Occasional lumbar punctures had been done during the first 2 weeks following the operation for drainage and for the relief of increased pressure. After purulent cerebrospinal fluid infected with Type 17 pneumococci was obtained, however, lumbar punctures were done 3 or 4 times daily. Procaine, 1 to 2 cc. of a 2% solution, was used for local infiltration of the skin and subcutaneous tissues prior to each puncture. Positive cerebrospinal fluid cultures were obtained intermittently during the first 10 days but the fluids obtained during the following 3 weeks were all sterile. During the last week of this period, the subcutaneous tissues over the 3d, 4th and 5th lumbar spaces, particularly the 4th, became soggy and tender. These were the spaces which had been used for the puncture. Increasing difficulty was encountered in entering the subarachnoid space due to underlying induration, but a free flow of clear fluid was obtained each time after the dura was entered. A sinus tract developed along the 4th lumbar space and this drained pus from which Type 17 pneumococci were obtained on culture. Fortunately, no epidural abscess developed and the cerebrospinal fluid did not become infected. When further punctures in this space were avoided, healing gradually occurred while sulfanilamide therapy was being continued. The patient was discharged much improved at the end of the 12th postoperative week.

CASE 2. A 67 year old man was admitted to the hospital in November, 1940, 10 days after a head injury. He was found to have purulent meningitis, and Type 19 pneumococci were obtained from cultures of blood and cerebrospinal fluid at entry. Treatment with sulfapyridine was begun promptly with an intravenous injection of its sodium salt followed by oral doses. Blood sulfapyridine levels were maintained at 9 to 11 mg. per 100 cc. during the 1st week and between 5 and 9 mg. thereafter. In addition, the patient received 330,000 units of Type 19 antipneumococcus rabbit serum on the day after entry.

Lumbar punctures were done daily during the first 12 days and again during the 4th week. Procaine, 2% solution, was used each time for infiltration of the skin and underlying tissues down to the dura. Blood cultures became negative but spinal fluid cultures continued to be positive for 6 days during which the protein content and leukocyte counts dropped steadily and the sugar level reached and remained normal. From the 7th to the 19th day cisternal fluid was sterile. On the 4th day the back over the site of the lumbar punctures became red and indurated and this gradually extended as a cellulitis to involve a large area of the back. Culture of the wound at the 4th lumbar space yielded Type 19 pneumococci.

The patient remained afebrile for about 10 days and seemed generally improved so the sulfapyridine was discontinued. Fever then recurred, as did the infection of the back, and the drug therapy was resumed after 3 days. During this time signs of meningitis also recurred and both the blood and cerebrospinal fluid cultures again became positive and its sugar content dropped. In spite of continued therapy with sulfapyridine and later with sulfadiazine, both orally and parenterally, the patient's fever and infection persisted and the patient died at the end of the 5th week.

CASE 3. A 66 year old man was admitted to the hospital in February, 1942, after a 4 weeks illness and was found to have an extensive but apparently resolving lobar pneumonia and purulent meningitis. Type 25 pneumococci were obtained from cultures of the blood and cerebrospinal fluid at the time of entry. Treatment with sulfadiazine was begun promptly and continued for 5 weeks during which the concentration of the drug in the blood was maintained between 10 and 15 mg. per 100 cc. In addition, 240,000 units of Type 25 antipneumococcus rabbit serum was given intravenously on the day after admission.

Lumbar punctures were done twice daily during the first 3 days, chiefly

for the relief of increased intracranial pressure, and at greater intervals thereafter to observe the progress of the meningeal infection. Procaine in a 2% solution was used each time to infiltrate the skin and underlying tissues down to the dura. Blood cultures taken on the day after entry and later were all sterile. Cultures of all the cerebrospinal fluids obtained during the first 48 hours were positive and subsequent ones obtained during the next 11 days showed no growth. During this time the protein and cellular content dropped almost to normal and the level of the sugar rose to normal and remained so for over a week.

At the end of the second week in the hospital when the temperature, pulse and white blood cell count had been normal for 10 days and the lungs had completely cleared, another lumbar puncture was done. The fluid was clear and colorless with a protein content of 62 and sugar of 46 mg. per 100 cc. and a leukocyte count of 50 cells per c.mm., of which 40 were lymphocytes. Culture of this fluid, however, yielded Type 25 pneumococci. It was then noted that there was a small, red, indurated area under the skin at the 5th lumbar space where all of the punctures had been done. Another puncture done in the 3d lumbar space after this result was obtained yielded fluid which was sterile, although para-aminobenzoic acid had been added to the culture medium. At this time and after subsequent punctures 1% sodium sulfadiazine in physiologic salt solution was injected along the path of the needle as it was withdrawn. The local lesion cleared and there was no further evidence of infection. The patient was discharged improved after an additional 6 weeks of observation.

CASE 4. A 16 year old girl was admitted to the hospital Nov. 11, 1941, with a history of a chill followed by fever, prostration and pleuritic pain of 1 day duration. The physical signs in the chest were consistent with fluid in the right pleural cavity, and the white blood cell count was 29,000 per c.mm. Sulfathiazole therapy in full doses was started promptly and continued without interruption for 5 weeks. During this time the blood levels of free sulfathiazole ranged between 2.2 and 4.1 mg. per 100 cc. in spite of additional doses of 3 gm. each of sodium sulfathiazole, intravenously given on several days.

A thoracentesis was attempted on the day after admission but yielded no fluid from three different sites. On the 6th day, however, another chest tap yielded 5 cc. of yellowish-green, odorless pus from which hemolytic *Staphylococcus aureus* was obtained in abundance and in pure culture. On the following day 15 cc. of similar fluid was obtained from a second tap. Procaine was used to infiltrate the skin and underlying tissues down to and probably including the parietal pleura at each point. During the next 3 days, small abscesses developed at the site of each of the puncture wounds and extended deep into the subcutaneous tissues. On the 11th day a thoracotomy and rib resection was performed with 1% pontocaine (which is not a p-aminobenzoic acid derivative) for local anesthesia. In addition to the abscesses in the thoracic wall, many loculated cysts were found extending into the interlobar fissure and down to the diaphragm. All contained thick pus from which *Staphylococcus aureus* was cultured. The pockets were broken up into a large single cavity into which 10 gm. of powdered sulfadiazine was scattered and an iodoform gauze pack inserted.

Cough productive of purulent sputum, fever and leukocytosis continued for another 4 weeks during which the cavity decreased in size. The patient was discharged at the end of the 8th week with a small healing cavity.

**Comment.** In each of the first 3 cases, procaine was repeatedly injected into the same areas. Infection, apparently conveyed from the subarachnoid space, developed along the track of the needle that was used to penetrate the dura. This infection developed in spite of the continuous presence in the body of bacteriostatic concentration of sulfonamide drugs. That effective sulfonamide bacteriostasis was maintained throughout the rest of the body at the time is

indicated in Cases 1 and 3 by the fact that there was no evidence of active infection of the spinal fluid when the organisms were obtained from the tissues and also by the failure of reinfection to occur when the dura was penetrated by the needle which had passed through the infected tissues. It is fair to assume that the sulfonamide bacteriostasis was inhibited in the tissues locally by the high concentration of procaine that was present at the site of infiltration and that this inhibiting action lasted long enough for infection to become established in that area. In Case 2 the local infection of the skin and subcutaneous tissues was extensive and may have been a source of reinfection.

In Case 4 infection with a hemolytic *Staphylococcus aureus*, which is generally less amenable to sulfonamide therapy, became established at each of the sites where procaine infiltration was carried out in attempting to do a thoracentesis. Infection from the underlying lung or pleura became established along the track of the thoracentesis needle in each instance.

This complication of procaine anesthesia may be avoided in one of two ways: either (1) a different drug may be used which is not of the p-aminobenzoic acid series<sup>11,13</sup> and that will not have the sulfonamide inhibiting effect; or (2) a small amount of sulfonamide drug may be injected from a different syringe through the same needle while it is being withdrawn. For most sulfanilamide derivatives 0.5 to 1% solution of the sodium salt in physiologic saline will serve the purpose.

With respect to the systemic sulfonamide inhibiting action of procaine absorbed from an area of local infiltration, it is fair to assume that this effect is not significant clinically except in rare cases. Procaine like p-aminobenzoic acid<sup>21</sup> is conjugated in the body and it is excreted in the urine in both the free and conjugated forms at a much more rapid rate than are any of the effective sulfonamides now in general use. This inhibiting action should, therefore, be of importance only in patients with severe infections in whom a delay of a few hours in the effective action of a sulfonamide drug may be crucial. When used in spinal anesthesia, the amount of procaine absorbed into the circulation and present in a form in which it might have an antisulfonamide action is probably very small indeed.<sup>9</sup> The high concentration of the free procaine in the spinal fluid, however, may be of importance, particularly if infection is accidentally introduced.

**Conclusions.** Procaine, in amounts ordinarily employed for local anesthesia, may be absorbed into the circulation in sufficient concentration to exert a definite inhibiting effect on the action of sulfonamide drugs that may be present in the blood.

Infection introduced into an area which has been infiltrated with procaine may become established locally in spite of the continuous presence in the body of bacteriostatic concentrations of sulfonamide drugs.

It is desirable to use local anesthetic drugs other than p-aminobenzoic acid derivatives for infiltration when performing exploratory punctures of potentially infected areas. Procaine, or similar anesthetics of the p-aminobenzoic acid series should also be avoided in extensive

operative procedures on patients having severe infections in which rapid and effective action of sulfonamide drugs is essential.

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## THE TOXICITY OF SULFADIAZINE

## OBSERVATIONS ON 1357 CASES

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THE greater clinical value of sulfadiazine over the other commonly used sulfonamide drugs depends in great measure upon its relatively lower degree of toxicity.<sup>2-4</sup> It has seemed important to us to record and evaluate the toxic reactions that have appeared in the patients treated with sulfadiazine or sodium sulfadiazine on all of the services at The New York Hospital. The chief advantage of this study is that every patient was seen daily by physicians on the alert for the occurrence of sulfonamide reactions, and the recognition of signs of toxicity was further aided by a hospital routine which included in almost all cases daily measurement of fluid intake and output, blood count,

urinalysis, and blood sulfadiazine determination. In addition there were frequent other blood chemical studies, routine Roentgen ray and bacteriologic procedures, and in many cases urologic and dermatologic consultations. We feel that very few toxic reactions were overlooked; on the other hand, all reactions included were well substantiated.

To May 1, 1942, we observed a total of 1357 patients treated with a daily dosage of 3, 4 or 6 gm. of sulfadiazine\* orally over a period of 2 days or more, or with at least 2.5 gm. or more daily of sodium sulfadiazine intravenously. Of these, 1113 received sulfadiazine orally and 244 received sodium sulfadiazine intravenously, either alone or supplementary to sulfadiazine by mouth. Of the patients receiving oral sulfadiazine, therapy lasted between 2 and 14 days in 973. However, 120 patients received sulfadiazine for more than 2 weeks, and 18 patients for more than 4 weeks. Twenty-nine patients each had more than 8 injections of sodium sulfadiazine intravenously. Patients of all ages were included (Table 2). Of the 1113 patients who were given sulfadiazine orally, 912 received a daily dosage of 6 gm., 86 received 4 gm., and 115, 3 gm. The infants and children included in this study were given a daily dosage of at least 0.1 gm. of sulfadiazine or sodium sulfadiazine per kilogram of body weight and therefore are included with the adults on the 6-gm. daily dosage. The 244 patients who were given sodium sulfadiazine intravenously received 2.5 gm. (10 cc. of 25% solution) every 8 to 24 hours, the time interval depending upon whether sodium sulfadiazine was used alone or supplementary to oral therapy.

Important factors which might be expected to influence the occurrence of toxic reactions are: (1) Daily dosage of drug. (2) Presence or absence of previous sulfonamide therapy (or previous reactions to the sulfonamides). (3) Total dose of drug and the length of time during which the drug is administered. (4) Route of administration. (5) Age of patient. (6) Presence or absence of impaired renal function. (7) Fluid balance. (8) pH of the urine.

Before determining the influence of these different factors, a baseline for the toxicity of sulfadiazine was created by ascertaining the incidence and severity of reactions under the most usual conditions of therapy—those that most often prevail in the hospital in the treatment of such conditions as pneumonia, meningitis, and puerperal and surgical infections. It is to be particularly noted that adjuvant alkali therapy was not given to these patients as a prophylactic measure against renal reactions. This treatment was instituted later and has proven effective in preventing renal reactions.<sup>5,6</sup> An analysis of these later cases compared with those included in this paper is being made separately.<sup>7</sup>

**Toxicity Under Usual Conditions of Therapy.** We found that there were 705 individual patients in our series (Column 3 of Table 1) who had received an oral dosage of sulfadiazine of 2 or 4 gm. initially, followed by 1 gm. every 4 hours continued for not less than 2 days or

\* The sulfadiazine for most of these patients was kindly supplied by Lederle Laboratories, Inc., Pearl River, N. Y.

more than 14 days, none of whom had had sulfonamide therapy previously and none of whom received sodium sulfadiazine intravenously. Patients of all ages were included in this group. The fluid output in most of these patients was well maintained; none of the patients had serious impairment of renal function.

Of the 705 patients, 62 (8%) exhibited some definite evidence of toxicity to sulfadiazine. The corollary, of equal interest and significance, is that 643 (92%) of the patients treated orally with a full therapeutic dosage of sulfadiazine showed no recognizable toxic manifestations. The much lower incidence of minor toxic effects following sulfadiazine are also impressive, as compared with those of sulfanilamide, sulfapyridine, or sulfathiazole. Practically all patients taking sulfapyridine are made "sick" by the treatment; if they are not severely nauseated and vomiting, at best they have no appetite, appear pale and gray, and are depressed and unhappy. During therapy with sulfanilamide and sulfathiazole, although severe vomiting is much less common than with sulfapyridine, the patients are frequently off-color and depressed, complaining commonly of nausea, headache, or other symptoms. The patient on sulfadiazine therapy is usually totally different. He usually takes the drug without the slightest complaint; nausea or loss of appetite is rare, his color is normal, he is not depressed. Long<sup>12</sup> has recently stated that the frequency of important toxic reactions following sulfadiazine is only 6.5% as compared with 11.9% following sulfanilamide, 15.9% following sulfapyridine, and 18.6% following sulfathiazole.

The most common toxic effect following sulfadiazine in the 705 patients (Table 1, Column 3) was renal irritation. This reaction occurred in 34 patients (4.8% of the group). Its significance becomes obvious when it is noted that of the 62 patients who showed signs of toxicity, 34 had those of renal irritation. Two of the 34 patients had fever also, and 1 both fever and a rash. Drug rash and fever occurred 14 times (2% of the 705 patients). Therefore, in this large series only 15 patients (2%) had any reaction other than renal irritation or rash and fever. The rise in temperature accompanying the drug rashes was sometimes only slight. Drug fever alone was recognized only once. A definite drop in the total number of white blood cells below 3000 per c.mm. or in the granulocytes below 35% was observed in 6 cases, but in none of these did the total white count fall below 2000 per c.mm., or the percentage of granulocytes below 10. In none of the patients were changes in the red blood cells recognized. In 1 of the surgical cases, however, a severe thrombocytopenia resulting in death occurred following 6 days of treatment with sulfadiazine in a dosage of 6 gm. daily. This was the only fatal toxic reaction in the total group of 1357 patients.\* Severe nausea and vomiting occurred 5 times, and there was 1 case of conjunctivitis and 1 of severe headache and vertigo.

\* Since this paper was written, an additional 1496 patients have been treated in The New York Hospital with sulfadiazine orally or sodium sulfadiazine intravenously without another fatality in the 2853 cases treated to date.



TABLE 1.—TOXIC REACTIONS AFTER SULFADIAZINE THERAPY

	Oral sulfadiazine—1113 patients					Sodium sulfadiazine I.V. with or without sulfadiazine orally*	Total patients
	2 or 4 gm. initially, followed by 6 gm. daily*						
	2 to 14 days inclusive						
Reaction	3 gm. daily* 115 patients 1	4 gm. daily* 86 patients 2	No previous sulfonamides 705 patients 3	Previous sulfonamides 87 patients 4	Over 14 days 120 patients 5		
Renal reaction . . . . .		4	31	4	3	16	58
Renal reaction with drug fever . . . . .		..	2	..	..	..	2
Renal reaction with rash and fever . . . . .		..	1	..	..	2	3
Drug rash and fever . . . . .		..	13	2	2	2	19
Drug rash with leukopenia . . . . .		..	..	1	..	..	1
Drug rash with peripheral neuritis . . . . .		..	..	..	..	1	1
Drug rash with stomatitis . . . . .		..	..	..	..	1	1
Leukopenia and/or granulocytopenia . . . . .		1	6	2	4	2	15
Drug fever alone . . . . .	1	..	1	1	1	..	4
Nausea and vomiting . . . . .		2	5	..	..	2	9
Thrombocytopenia . . . . .		..	1†	..	..	..	1†
Jaundice—possible hepatitis . . . . .		..	..	..	..	2	2
Conjunctivitis . . . . .		..	1	..	..	..	1
Stomatitis . . . . .		..	..	..	..	1	1
Headache and vertigo . . . . .		..	1	..	..	..	1
Encephalopathy . . . . .		..	..	..	..	1	1
Arthralgia . . . . .		1	..	..	..	..	1
Total patients with reactions . . . . .	1	8	62	10	10	30	121
Per cent with reactions . . . . .	0.8	0.2	8.0	11.5	8.3	12.3	8.0

*Summary*

Total patients with renal reactions . . . . .	4 (4.7%)	34 (4.8%)	4 (4.8%)	3 (2.5%)	18 (7.4%)	63 (1.6%)
Total patients with drug rash . . . . .	..	14	3	2	6	25
Total patients with drug fever . . . . .	1	..	1	1	1	7
Total patients with W.B.C. effects . . . . .	1	6	3	4	2	16
Total patients with misc. reactions . . . . .	2	8	..	..	8	18

\* All patients treated for at least 2 days.

† This was the only fatal toxic reaction in the series.

**Influence of Daily Dosage of Drug.** For an evaluation of the rôle played by daily dosage of drug upon the toxicity of sulfadiazine, we have for comparison with the 6-gm. per day series 2 groups of cases, each treated with a lower dosage. In 1, the patients received 4 gm. daily (Table 1, Column 2), and in the other, 3 gm. daily (Column 1). The 86 patients given 4 gm. each day showed approximately the same percentage of reactions as did those who were given 6 gm. daily. However, it happened that none of the 86 patients developed a drug rash, and there was 1 case of arthralgia, which is one of the rare toxic conditions due to sulfonamide therapy.

That the incidence of toxic reactions is influenced by the daily dosage of sulfadiazine is indicated, however, by the fact that in 115 hospital patients receiving 3 gm. per day there was only one toxic reaction, a moderately severe drug fever that occurred on the 10th day of therapy and without previous use of the sulfonamides. Further evidence that there is a very low degree of clinical toxicity following a

3-gm. per day dosage of sulfadiazine comes from our observations on approximately 200 ambulatory patients not included in Table 1. Each of them received the 3-gm. amount for a period of 4 days and had at least 1 urinalysis and 2 blood counts during the time of therapy. In this group we discovered 1 case of mild granulocytopenia and 3 of mild renal irritation. While these findings show a very low degree of toxicity, they also indicate that reactions can occur with such a low dosage of sulfadiazine, and they present another warning against the overenthusiastic and careless use of the sulfonamides.

**Concentration of Sulfadiazine in the Blood.** The toxic reactions in 1155 patients who had frequent measurements of blood levels have been analyzed according to the concentration of drug in the blood. Of 117 patients showing less than 5 mg. per 100 cc. of sulfadiazine in the blood, 1.8% developed toxic reactions; of 505 having 5 to 10 mg. per 100 cc. concentration, 7% showed reactions; of 371 with 10 to 15 mg. per 100 cc., 12.8% had toxic symptoms; and of 162 patients with more than 15 mg. per 100 cc., 14.8% showed evidences of toxicity.

The 3-gm. daily dosage of sulfadiazine gave concentrations in the blood of 5 mg. per 100 cc. or less in 46% of the patients, of 5 to 10 mg. per 100 cc. in 36%, of greater than 10 mg. per 100 cc. in 18%. The corresponding values for the 4-gm. daily dosage were only slightly higher. On the other hand, with the 6-gm. daily dosage the concentration in the blood was less than 5 mg. per 100 cc. in only 5.8%, between 5 and 10 mg. per 100 cc. in 48.7%, and greater than 10 mg. per 100 cc. in 45.5%.

**The Effect of Previous Sulfonamide Therapy.** Our observations confirm those of Finland and his co-workers<sup>3</sup> and do not prompt the degree of concern that has been registered by some clinicians<sup>13,17</sup> regarding the dangers of repeated courses of sulfonamide therapy. Eighty-seven of our patients given 6 gm. of sulfadiazine daily had had previous sulfonamide treatment (Table 1, Column 4), and 10 of them (11.5%) had reactions, compared with 8% of the 705 patients similarly treated but without sulfonamides at a previous time. This increased incidence of toxic reactions seems to be significant because of the slightly higher incidence of drug rash and white blood cell effects; the incidence of renal complications was the same in both series.

We have observed a variety of relationships between reactions and previous sulfonamide treatment. For example, 1 patient developed an acute febrile reaction after a test dose of each of the sulfonamides: sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine. In another patient, first sulfapyridine, then sulfathiazole, and finally sulfadiazine produced a drug rash and fever. In the case of drug rashes, fever, and blood dyscrasias, a possible sensitization phenomenon is more likely than in the case of a reaction such as a renal complication, which is apparently most frequently, if not always, of a mechanical or chemical nature.

Our cases show a few instances in which sulfadiazine was well tolerated by patients who had previously manifested toxic effects from sulfathiazole, and many examples of a similar type when sulfadiazine

was administered to patients who had shown a marked intolerance to sulfanilamide or sulfapyridine. The reverse of this—tolerance to the other sulfonamides after intolerance to sulfadiazine—occurred but was less common.

No definite rules can be made for subsequent sulfonamide treatment in patients who have exhibited evidences of intolerance to sulfonamides, except that in such cases one always must be doubly cautious. It is true that most of the fatalities following the sulfonamides have occurred after some unheeded warning. In 1 instance, a patient treated with sulfathiazole had had a drug rash and fever 2 weeks before the occurrence of acute agranulocytosis and death. Another case, reported in a previous communication,<sup>14</sup> of fatal aplastic anemia and toxic hepatitis following sulfapyridine was preceded several weeks by drug fever. Many such cases might be cited. We have, on the other hand, treated with great caution but successfully patients with past records of granulocytopenia, rashes, and fever following the sulfonamides. In most instances there has been no repetition of the previous reaction. This practice is dangerous and should be avoided except where the treatment is urgent.

**Total Dose of Drug and Length of Time Administered.** One hundred and twenty patients were treated with sulfadiazine for more than 14 days (Table 1, Column 5), with 8.3% of reactions compared with 9.1% in the entire group of 792 patients receiving the same daily dosage of sulfadiazine but for less than 14 days (Columns 3 and 4). Drug rash, drug fever, and toxic effects on the white blood cells rarely occurred before the 5th day of either oral or intravenous treatment. Following oral therapy, renal irritation rarely occurred before the 4th day, but following sodium sulfadiazine intravenously it often appeared on the 2d or 3d day, and in 1 instance on the 1st day. Our data do not suggest that the patient who has been receiving the drug for more than 3 to 4 weeks is safe from toxicity, as has sometimes been stated, any more than that a short course of therapy is entirely safe. An important rule to follow in the use of the sulfonamides is to discontinue the drug as soon as the danger of toxicity outweighs its usefulness. It is true that in an individual case the longer the drug is continued the greater is the chance of reaction in that case. It is not good practice to continue sulfonamide therapy indefinitely in obscure infections in which the value of the drug is at best questionable.

We have treated patients for exceptionally long periods of time. One patient with subacute bacterial endocarditis received a total dosage of 1333 gm. of sulfadiazine, and 4 others with the same condition received between 400 and 500 gm. continuous treatment with decreasing dosage of drug extending for periods as long as 4½ months. These patients who received the drug over periods of several months did not show any unusual toxic manifestations due to the prolonged treatment.

**Route of Administration of Drug.** The use of the sodium salt of sulfadiazine intravenously has been a life-saving measure in many of the patients who have been unable to take sulfadiazine orally or have been in desperate need of immediate optimal treatment, but the

dangers of toxic reactions are greater. In 244 patients who were given 1 or more intravenous injections, 30 (12.3%) had some form of reaction as compared with 8.9% of reactions in 912 patients treated orally with 6 gm. daily. More striking than the difference in the total reactions is the difference in the renal reactions with 18 occurrences (7.4%), in the 244 patients treated intravenously, as compared with 41, (4.5%) in 912 patients treated orally. Also, there were 2 cases of mild and transitory jaundice with possible toxic hepatitis probably attributable to the intravenous therapy, whereas in the 912 cases of oral therapy there was no such occurrence.

**Age of Patient.** The age distribution of patients having toxic reactions is shown in Table 2. The most significant finding is a lowered incidence of reactions in the group less than 1 year of age. A greater tolerance for the sulfonamides by infants than by adults has been suggested. According to our analysis, the decreased incidence of total toxicity in infants is accounted for by a low incidence of renal reactions. Whether renal complications are less frequent in infants, or are not readily recognized, cannot be stated. Conceivably, increased alkalinity of the urine of infants compared with the urine of adults may result from dietary differences or more frequent vomiting, thereby reducing renal toxicity.

TABLE 2.—AGE DISTRIBUTION OF PATIENTS WITH TOXIC REACTIONS

Age in years	No. of patients	No. of patients with reaction	Percentage with toxic reaction
0- 1 . . . . .	127	5	3.9
1- 5 . . . . .	82	8	9.8
5-10 . . . . .	44	6	13.6
10-20 . . . . .	100	14	14.0
20-60 . . . . .	805	72	8.9
60-80 . . . . .	199	16	8.0
	<hr/> 1357	<hr/> 121	

**Renal Reaction and the Influence of Impaired Renal Function, Fluid Balance, and pH of Urine.** Our table shows that following sulfadiazine therapy, renal irritation is by far the most common reaction, occurring in 4.6% of all cases, and meaning that more than half of all the patients having any reaction at all have a renal complication. This toxic reaction is especially common after intravenous sodium sulfadiazine, occurring in 7.4% of all the patients treated. Recent publications have also emphasized the importance of this reaction and have described a number of resultant fatalities.<sup>1,8,9,11,15,16</sup>

The particular manifestations of renal irritation in the 63 patients in whom this reaction was observed are shown in Table 3. Nineteen patients had microscopic hematuria alone which could be accounted for only on the basis of the sulfonamide therapy. Nine patients had grossly blood urine. Pain alone occurred in 8 patients. In 13 cases pain was accompanied by microscopic hematuria and in 10, by gross hematuria. The distribution of oliguria in respect to other evidences of renal complication is listed in Table 3. The renal pain experienced was of several types. More commonly it was flank pain and tenderness

similar to that of renal infarct. Typical ureteral colic occurred in some of the cases, and in a few the pain and tenderness were of a severe, generalized abdominal type suggesting appendicitis or mesenteric thrombosis. Oliguria was definitely recognized 7 times, and was most common when the other symptoms were most severe, although in 1 instance it occurred without any other finding. Anuria developed in only 2 cases, and in neither of these did it exist for more than 24 hours. All of our patients who experienced renal complications recovered without treatment other than cessation of sulfadiazine therapy, forcing of fluids, and administration of alkali. In no instance was it necessary to resort to ureteral catheterization and lavage. None of the patients who had histories of renal complications from sulfadiazine has been found to have any permanent renal impairment due to the sulfadiazine therapy.

TABLE 3.—MANIFESTATIONS OF RENAL IRRITATION

	No. of patients	No. with oliguria
Microscopic hematuria . . . . .	19	0
Gross hematuria . . . . .	9	2
Pain . . . . .	8	1
Pain and microscopic hematuria . . . . .	13	1
Pain and gross hematuria . . . . .	10	2
Oliguria only . . . . .	1	1
Total . . . . .	63	7

Seven of the 63 patients who had had renal irritation later die of other causes and were examined at necropsy. Of these, 6 showed renal lesions attributable to the toxic effect of sulfadiazine, consisting of concretions of drug in the renal pelvis and deposits of drug within the renal parenchyma with tubular degeneration and associated interstitial inflammatory reaction and occasionally secondary degeneration of the nephron. The kidneys of 39 patients who died during or soon after treatment with sulfadiazine, in whom renal irritation had not been recognized during life, were also examined at necropsy and pathologic changes like those described above were observed in only 3. In none of the 9 patients who showed at necropsy renal damage attributable to sulfadiazine were the lesions sufficiently extensive to justify the belief that permanent and serious impairment of renal function would have ensued had the patient lived.

While impaired renal function may have an influence upon the incidence and seriousness of renal reactions following the use of sulfonamides, of greater clinical importance is the effect of sulfonamide irritation of the urinary tract upon the prognosis in renal disease. The nature of the pathologic changes that occur in the kidneys following sulfadiazine therapy would strongly suggest that their superimposition upon those already caused by severe renal disease would aggravate the primary condition and possibly produce or make more serious a uremic condition. A review of the fatal kidney reactions that have been reported in the literature following sulfadiazine therapy<sup>9</sup> and also sulfathiazole and sulfapyridine reveals that in a number of them this was apparently the nature of the death. Our present series includes

none of these cases; the drug has been used in patients with renal insufficiency only when there was great need for it, and at times in reduced dosage as indicated by the blood sulfadiazine levels.

The pH of the urine definitely influences the incidence of renal reactions following sulfadiazine which in most, if not all, cases is due to the precipitation of sulfadiazine compounds in the kidneys and urinary tract. Recently it has been shown<sup>5,6,10</sup> that the solubilities of sulfadiazine and particularly of  $N_4$ -acetylsulfadiazine, the compound usually precipitated in the urine, increase markedly with increasing pH within the physiologic pH range of urine. For example, 512 mg. of  $N_4$ -acetylsulfadiazine can be dissolved in 100 cc. of buffer solution at pH 7.5 compared with only 18 mg. per 100 cc. at pH 5.<sup>6,7</sup> Furthermore, crystalluria, evidences of renal irritation or of urinary tract obstruction, were not encountered in approximately 200 patients in whom the urine was maintained neutral or alkaline by adjuvant alkali therapy during treatment with 6 gm. daily or more of sulfadiazine orally, or with sodium sulfadiazine intravenously.<sup>7</sup> On the basis of clinical studies, 15.6 gm. daily of sodium bicarbonate (or amounts of other organic sodium salts with equivalent available base excess), divided into 6 doses of 2.6 gm. (40 gr.) administered every 4 hours, is recommended as the dosage usually appropriate to maintain the urine neutral or slightly alkaline during sulfadiazine in adults. We did not observe any gastro-intestinal upsets or other undesirable effects from this dosage of sodium bicarbonate. In the instance of patients suffering from heart failure or impaired renal function, it has been advocated that sodium salts be used cautiously.

We do not consider the maintenance of a large daily volume of urine to be as important a safeguard against the renal reaction as it seems generally to be thought. One-fifth of our 63 patients who had the renal reaction had never had a 24-hour urine volume of less than 1000 cc., and in several the reaction occurred although the output of urine had always been more than 2000 cc. daily. We mention this point chiefly to demonstrate that forcing fluids alone is often insufficient and to emphasize the additional need and efficacy of alkali therapy.

**Summary and Conclusions.** 1. The toxic reactions that occurred in 1357 hospital patients treated with sulfadiazine orally, or with sodium sulfadiazine intravenously alone or in combination with sulfadiazine orally, are tabulated and analyzed. In this entire group there was 1 fatality, a case of thrombocytopenic purpura which was attributed to sulfadiazine.

2. Eight per cent of 705 patients, who received 6 gm. of sulfadiazine daily for at least 2 days and not more than 14 days, showed evidences of toxicity. The renal reaction was the most frequent single toxic manifestation.

3. With intravenous sodium sulfadiazine the incidence of renal reactions was almost doubled, and thereby the total incidence of reactions was raised.

4. The effect of previous use of sulfonamide therapy upon toxicity was observed in 87 patients, and in these there was a slightly increased

incidence of reactions attributable to a slight increase in drug rash, drug fever, and leukopenia.

5. The renal complications comprised more than half of the toxic reactions following sulfadiazine in the entire series. With the indication that this reaction can be prevented by proper fluid intake, together with appropriate alkali therapy, the total incidence of toxic reactions from sulfadiazine can be reduced to approximately 4%.

6. This study indicates a decided superiority of sulfadiazine over the other commonly employed sulfonamides on the basis of a low degree of clinical toxicity.

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#### GARGOYLISM

#### REVIEW OF LITERATURE WITH REPORT OF TWO CASES

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IN 1936, Ellis, Sheldon and Capon<sup>2</sup> described under the name of "Gargoylism" a syndrome complex which is characterized by chondrodystrophic skeletal changes and deposition of a lipid-like substance in many of the body tissues. The name "Gargoylism" emphasizes the large head, grotesque, inhuman facies, and the deformed limbs which "suggest the appearance of gargoyles," making the patients

look much alike. It has also been referred to as Hurler's Syndrome, Dysostosis Multiplex and Lipochondrodysplasia. Although Hunter, in 1917,<sup>3</sup> was the first to call attention to this condition, Hurler, in 1919,<sup>4</sup> was the first to describe the disease as a separate clinical entity.

In spite of the fact that the disease is easily recognized only a comparatively few cases (50)<sup>1</sup> have so far been reported in the world's literature. This is an indication of its rarity.

The following is a report of 2 cases studied at the Child Guidance Home:

**Report of Cases.** CASE 1. A. B., a white boy, was referred to the Child Guidance Home at the age of 7 years and 8 months for determination of his mental status. According to the mother, the boy had been showing a gradual mental deterioration since the age of 4½ years. She stated that the boy had recited nursery rhymes at the age of 2½ years, articulating well enough to be understood. Two years later, this interest in the rhymes began to wane and his speech became indistinct. It is the mother's feeling that deterioration, as manifested by progressive indifference to mental stimulation, has progressed steadily since that time.

The family history showed that there was no blood relationship between the parents, neither was there a history of epilepsy, mental or nervous diseases, alcoholism, glandular disturbances, feeble-mindedness, or of congenital defects in the parents or collaterals.

The patient was the oldest of 2 brothers. He was born to a primipara aged 24. Delivery was instrumental. He weighed 7 pounds and 2 ounces at birth. He was an extremely thin baby. Dentition began at 6 months. He walked at the normal time and began to talk at 1 year. Nutrition, sleep and excretory habits were normal up to that time. He suffered mild attacks of measles, whooping cough and chickenpox in early infancy. At 17 months he was hospitalized because of an increasing deformity of the spine. Roentgenographic examination of the spine at that time revealed an atypical destructive lesion of the 1st and 2d lumbar vertebræ and a diagnosis of spondylitis of unknown origin was made. During his stay at the hospital, it was noted that the boy already had coarse features, thick wrists, large hands, and a very large head. He was examined several months later because of an apparently increasing hydrocephalus. Additional roentgenograms revealed retarded ossification; the spine showed no changes from those previously noted. The sutures of the skull were firmly closed, and the bones of the skull seemed thicker than normal. Except for a marked hearing loss, the other findings were negative.

Physical examination revealed a good nutritional state. The head was large with occipito-parietal flattening; the bossæ were prominent; the face was large and expressionless; the orbits were wide set. There was marked puffiness of the upper eyelids. The nose was saddle-shaped, with wide nares. The lower mandible appeared to be smaller than normal and was misshapen. The lips were thick. The upper teeth were missing, while the lowers were markedly spaced, irregular, peg-shaped, and notched. The general impression was one of grotesqueness and unreality. The boy's posture was also characteristic; he stood with his knees slightly flexed; all the other joints, especially the elbow joints, were also held in a flexed position, giving the child a very awkward appearance. The head was retracted backward and rested on the shoulders, which were slightly rounded. There was a marked thoracolumbar kyphosis and a slight lumbar scoliosis to the right. As a result, the abdomen protruded and resembled a pot belly. The hands were small, but broad, with short tapering fingers. Incurving of all the fingers was also noted.

The boy was 4 inches under height for his age. His weight was normal for his age and height. His measurements were as follows: The circumference of the skull was 21½ inches (55 cm.), bitemporal 13½ inches (34.5 cm.), torso



16½ inches (42 cm.), upper extremities 18 inches (46 cm.), lower extremities 26 inches (66 cm.), vertex to symphysis 21½ inches (55 cm.), symphysis to floor 21½ inches (55 cm.), span 39 inches (99.5 cm.), chest 24 inches (61 cm.), waist 26 inches (66 cm.), and hips 27 inches (69 cm.).

There were no undue deposits of fat, although the boy was rather plump. The skin on the face was rather smooth, while the skin on the rest of the body was dry and rough. The hair on the head was dry and coarse. There was marked hypertrichosis of the extremities and of the back. The thyroid gland was not enlarged.

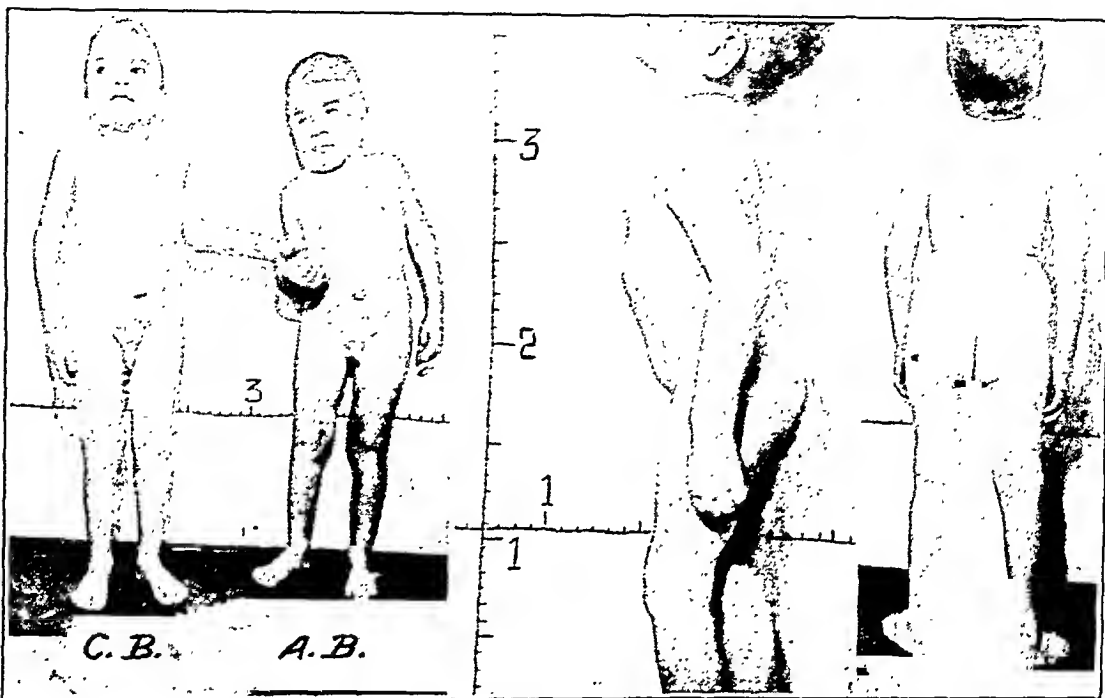


FIG. 1

FIG. 2

FIG. 3

FIG. 1.—C. B. at the age of 6 years 8 months, and A. B. at the age of 7 years 8 months. Note the very typical posture with the retracted head, the short neck, pot belly, tendency to umbilical hernia and the flexed joints.

FIG. 2.—Side view of C. B. at the age of 6 years 8 months. Note the retracted head, the short neck, the rounded shoulders, the thoracolumbar kyphosis, the pot belly, and especially the flexed position of the elbow and knee joints. (Figures on vertical line represent height in feet.)

FIG. 3.—Rear view of A. B. at the age of 7 years 8 months. Note the retracted head, the short neck, the rounded shoulders, the thoracolumbar kyphosis, and the flexed elbow joints. (Figures on vertical line represent height in feet.)

The chest was barrel-shaped. The heart and lungs were negative to percussion and auscultation. There was a marked umbilical hernia, the ring easily admitting 2 fingers. Both the liver and spleen were greatly enlarged. The lower edge of the liver was palpable about 4 finger-widths below the right costal margin. The edge of the spleen was easily palpable just below the level of the umbilicus. The genitalia were small, both testes being in the external rings, which were very large. No secondary sex characteristics were present.

The neurologic examination was negative, except for the absence of the deep tendon reflexes, and the patient's insensitiveness to pain. Bone conduction was normal.

The ophthalmologist reported as follows: "The eye movements were parallel and normal in the cardinal directions. The pupils reacted normally to light, accommodation and consensually. Visual acuity was 20/30 in each eye. The

corneæ, lenses, and mediæ were normal; the fundi were negative; and the intra-ocular tension was apparently normal."

The otologist reported as follows: "Both drums could not be seen because of cerumen. Bone and air conduction were apparently decreased on both sides. On the audiometric test a hearing loss of over 50% in both ears was noted."

The roentgenographic findings were as follows: "A-P and lateral views of the skull showed an increase in size. The skull was thickened, and the sphenoid bone was unusually large. The sella turcica was larger than normal, but in proportion to the increased size of the skull. The paranasal sinuses were clear. A-P view of the chest showed the great vessels normal in position, shape and size. The heart was somewhat enlarged to both sides. The lung fields were clear. The diaphragm was somewhat elevated. A-P and lateral views of the thoraco-lumbar spine showed a subluxation of the 2d and 5th lumbar vertebræ

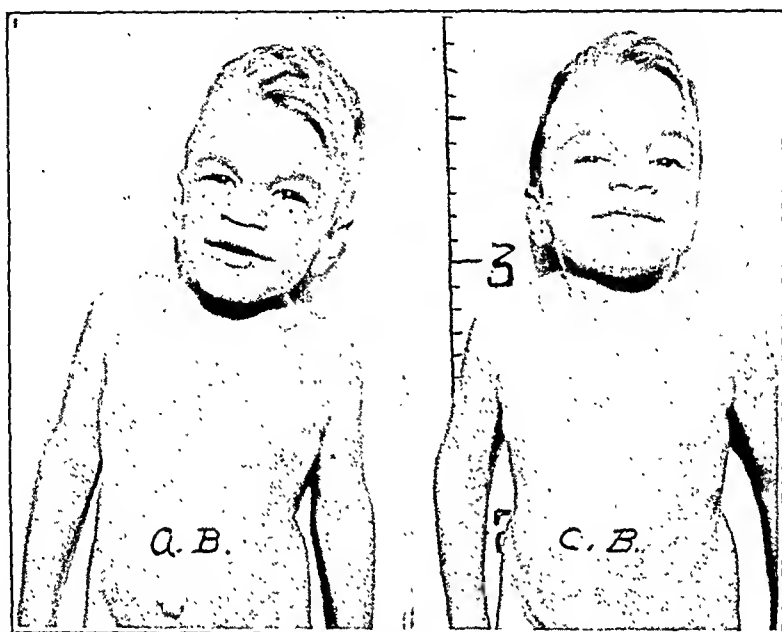


FIG. 4.—Note marked evidence of gargoylism in the two brothers. (Figures on vertical line represent height in feet.)

which were greatly reduced in size. The anterior margins of the bodies of the vertebræ were rounded. The bone age as determined from the ossification centers was 4 years, giving the child a bone quotient of 50.<sup>5</sup> The hands especially showed marked shortening of the bones, which were very broad and thick. There was tufting of the terminal phalanges and marked incurving of the little fingers. All the bones showed poor mineralization." (Figs. 5, 6 and 7.)

The blood count showed 4,560,000 red blood cells, 9300 white cells and 300,960 platelets. Hemoglobin was 13.3 gm. The differential count revealed 38% neutrophils, 49% lymphocytes, 8% monocytes, 4% eosinophils and 1% basophils. There was slight anisocytosis and slight polychromasia. Occasional stippled red cells were seen, and some of the monocytes appeared abnormal.

The blood serologic tests were negative. The sedimentation rate (Cutler) was 24 mm./60 min. The coagulation time was 2 minutes; the bleeding time was 3 minutes. The prothrombin time (Quick) was 23 seconds. Chemical values: fasting blood sugar 76 mg., N.P.N. 27.3 mg., urea N 10 mg., creatinine 1.4 mg., calcium 11 mg., phosphorus 4.5 mg., phosphatase 4.7 units (Bodansky), chloride 607 mg. as NaCl, cholesterol 132 mg., total protein 7.24 mg., albu-

FIG. 5

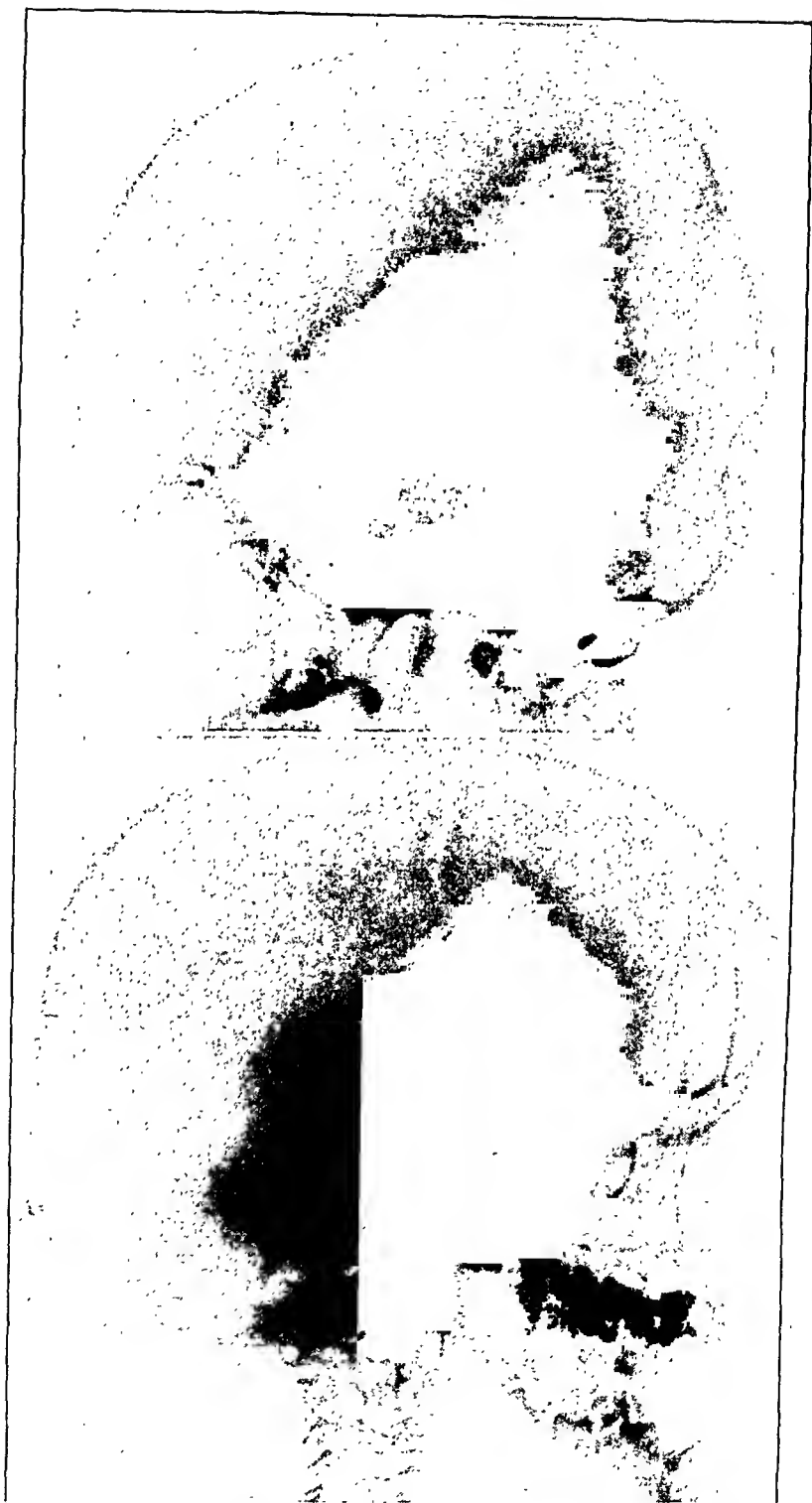


FIG. 8

FIG. 5. Case 1. (A. B.) Roentgenogram of skull showing flattening of occipital region, prominent frontal bossæ, and slightly enlarged sella turcica. Skull as a whole is enlarged.

FIG. 8.—Case 2. (C. B.) Note the large and thick skull with prominent frontal bossæ.

min 4.64 gm. and globulin 2.6 gm. The blood diastase was 137 units (Somogyi). The direct van den Bergh reaction was negative, while the indirect reaction showed a trace of bilirubin. The icteric index was 10.

The urinalysis was negative. The urine diastase determination showed 20 units of diastase. The hippuric acid liver function test (Quick) showed an output of 2 gm. of benzoic acid in 4 hours.

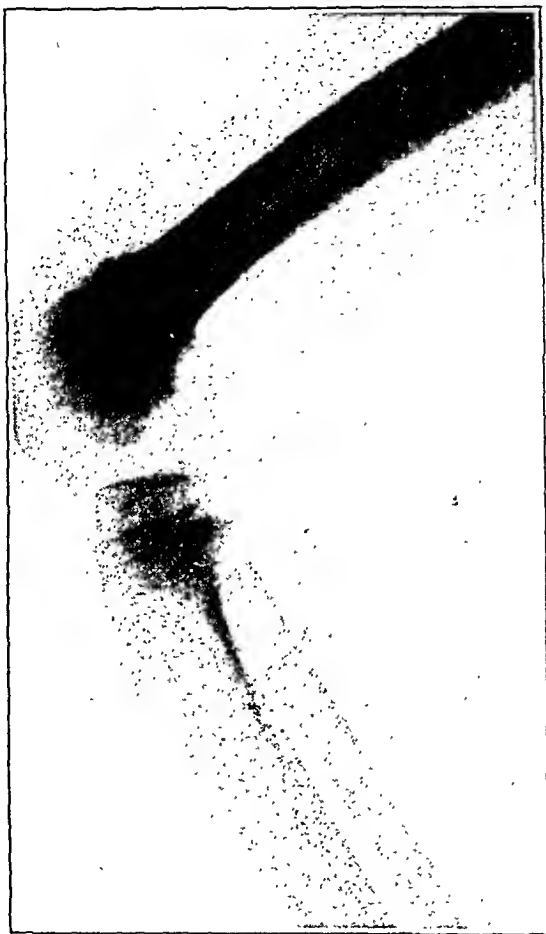


FIG. 6.—Case 1. (A. B.) Note marked incurving of all the long bones, especially the tibia.

The tuberculin tests, both P.P.D. No. 1 and P.P.D. No. 2, were negative.

Except for sinus arrhythmia, the electrocardiogram was within normal limits.

An attempt was made to obtain an electro-encephalogram, but because of the patient's uncoöperativeness, this could not be carried out.

On the revised Stanford Binet Test, Form L, the boy made a mental age of 2 years, thus giving him an Intelligence Quotient of 25. He was also tried on the Seguin Form Board, but made no score. On the Vineland Social Maturity Scale, the boy obtained a Social Quotient of 55.4.

The boy's behavior during his stay at the Home was extremely immature and characteristic of a markedly intellectually retarded child. He was very pleasant and coöperative to the best of his ability, which, however, was definitely limited. He was unable to carry out simple directions and needed constant supervision. His speech was guttural, muttered, and indistinct.

His memory for both past and recent events was very poor. He was dependent on others for his personal needs, being unable to dress or undress him-



FIG. 7.—Case 1. (A. B.) Note subluxation and reduction in size of 2d and 5th lumbar vertebræ; also the peculiar roundness of the anterior margins of the bodies of all the vertebræ.

self. He could not even feed himself properly. His reactions were entirely those of a feeble-minded child, no psychotic manifestations being present.

CASE 2. C. B., a brother of A. B. (Case 1), was 6 years and 9 months old when first seen at Child Guidance Home. Like his older brother, he was referred for determination of his mental status. The boy was attending the first grade in a public school, where he was not making satisfactory progress. The mother also noted that the boy was beginning to present symptoms similar to those of his brother.

The birth and delivery were uneventful. His birth weight was 8 pounds. He teethed at the normal time, began to talk at the age of  $1\frac{1}{2}$  years and started to walk when he was about 2 years old. Nutrition, sleep and excretory habits were normal. Like his brother, he suffered from measles, whooping cough and chickenpox during his early infancy. He had never been hospitalized, except for a few days when a tonsillectomy was performed. The mother noted that the boy was subject to colds and upper respiratory infections and that his hearing seemed to be greatly affected when he had a cold.

The boy had identically the same features as his brother. His head was very large with definite occipito-parietal flattening. There was bulging of the forehead with prominent bossæ. The face was large and expressionless. The orbits were widely spaced. A typical saddle-shaped nose and flaring nares were present. The lips were thick, especially the lower. The teeth were widely spaced, and somewhat peg-shaped. The tongue was large, but otherwise normal. His posture was identically the same as that of his brother. He stood with his knees slightly flexed. All the other joints, especially the elbow joints, were also held stiffly flexed, giving the child a very awkward appearance. The head was retracted backward and rested on the shoulders, which were slightly rounded. There was a marked thoracolumbar kyphosis and a slight lumbar scoliosis to the right. As a result, the abdomen protruded and resembled a pot belly. The hands were small, but broad with short tapering fingers. Incurving of all the fingers was present.

He was  $2\frac{1}{4}$  inches under height for his age and 6 pounds overweight for his height and age. The measurements were as follows: The circumference of the skull was 22 inches (56 cm.), bitemporal 14 inches (36 cm.), torso 15 inches (38.5 cm.), upper extremities  $18\frac{1}{4}$  inches (46.5 cm.), lower extremities  $26\frac{1}{2}$  inches (67.5 cm.), vertex to symphysis 22 inches (56 cm.), symphysis to floor 21 inches (53.5 cm.), span  $43\frac{1}{4}$  inches (110.5 cm.), chest 25 inches (64 cm.), waist 27 inches (68.5 cm.), and hips 28 inches (71.5 cm.). The boy was plump, but there were no undue deposits of fat. The skin on the face was smooth, while on the rest of the body, it was rough and dry. The hair was dry and coarse. There was marked hirsutism of the extremities and of the back. The thyroid gland was not enlarged.

The chest was barrel-shaped. The heart was not enlarged. However, there was a rough high-pitched systolic murmur, which was heard best in the 3d and 4th intercostal spaces to the left of sternum. The lungs were clear both to percussion and auscultation. The abdomen was protuberant with a large umbilical hernia. The ring easily admitted 2 fingers. Both the liver and the spleen were markedly enlarged. The liver edge was palpable about  $2\frac{1}{2}$  finger-widths below the right costal margin, while the edge of the spleen was palpable just below the left costal margin. The genitalia were small. Both testes were still undescended, but could be palpated in the inguinal canals. There was a small left inguinal hernia present. No secondary sex characteristics were present.

The neurologic examination was essentially negative.

The report of the ophthalmologist was as follows: "The eye movements were parallel and normal in the cardinal directions. The pupils reacted normally to light, accommodation and consensually. The visual acuity was 20/30 in each eye. The corneæ, lenses, and mediæ were normal. The fundi were negative. Intraocular tension was apparently normal."

The otologist reported as follows: "Both drums were dull and slightly injected, especially the right. Bone and air conduction were slightly decreased on both sides. There also was an apparent hearing loss in both ears as measured on the audiometer. The exact amount could not be determined."

The roentgenographic findings were as follows: "A-P and lateral views of the skull showed a marked increase in the size of the skull with thickened skull



FIG. 9.—Case 2. (C. B.) Note thickened and broadened metacarpals and phalanges with terminal tufting. Also incurving of all fingers. Bone age 3 years.

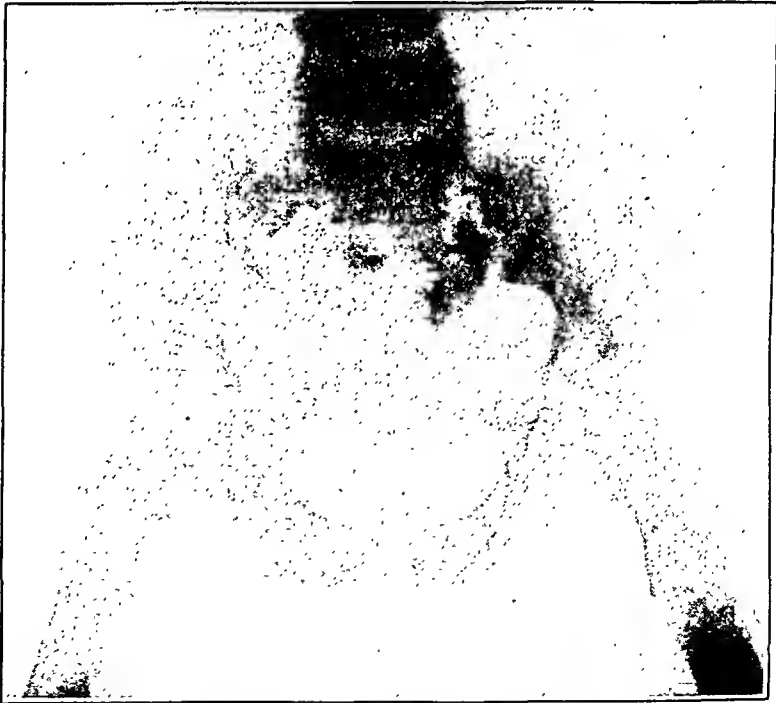


FIG. 10.—Case 2. (C. B.) Note lack of development of bones of pelvis and lower extremities in contrast to those of upper extremities and skull.

bones. The sutures of the skull were firmly closed. The lower jaw appeared to be out of proportion to the size of the skull. The sella turcica was moder-

ately enlarged but normal in proportion to the increased size of the skull. The paranasal sinuses were clear. Both lung fields were clear. The heart appeared to be slightly enlarged to both sides. The chest as a whole appeared to be very small. The bones of the thoraco-lumbar spine showed roundness of the anterior margins of the bodies of the vertebræ. The pelvic bones and joints were apparently normal. The child's bone age as determined from the ossification centers was 3 years, the bone quotient being 43%.<sup>5</sup> The hands showed shortening of the bones which was very broad and thick. There was marked tufting of the terminal phalanges and marked incurving of the little fingers. The mineralization of all the bones appeared to be poor." (Figs. 8 to 11.)



Fig. 11.—Case 2. (C. B.) Note the peculiar roundness of the anterior margins of the bodies of all the vertebræ.

The blood count showed 4,480,000 R.B.C., 7100 W.B.C., and 318,080 platelets. The hemoglobin was 12.8 gm. The differential blood count showed 48% neutrophils, 31% lymphocytes, 15% monocytes and 6% eosinophils. There was slight polychromasia present and some of the mononuclear cells appeared abnormal. Occasionally stippled red cells were seen.

The blood Wassermann, Kahn, and Kline tests were negative. The blood sedimentation rate (Cutler) was 17 mm./60 minutes. Coagulation time was 2 minutes, while the bleeding time was 3 minutes, 30 seconds. The prothrombin time (Quick) was 22 seconds. Chemical values: fasting blood



sugar 76 mg., N.P.N. 28.8 mg., urca N 9.8 mg., creatinine 1.4 mg., calcium 11.1 mg., phosphorus 4.7 mg. The phosphatase was 4.6 units (Bodansky), chloride 624 mg. as NaCl, cholesterol 123 mg., total protein 7.19 gm., albumin 5 gm. and globulin 2.19 gm. The blood diastase was 82 units (Somogyi). The direct van den Bergh reaction was negative, while indirect reaction showed a trace of bilirubin. The icteric index was 6.

The urinalysis was normal. The urine diastase showed an output of 10 units. The hippuric acid test for liver function (Quick) showed an output of 1.9 gm. of benzoic acid in 4 hours.

The tuberculin tests, both P.P.D. No. 1 and P.P.D. No. 2, were negative.

The electrocardiographic record was within normal limits.

An electro-encephalogram was made. Routine 6 lead bipolar and monopolar tracings were taken. These revealed a somewhat irregular record with, however, a frequency ranging from 6 to 9 per second. Alpha frequencies were best developed in the parietal and the occipital regions. There was no evidence of any focal abnormalities. The record as a whole appeared to be normal.

On the revised Stanford Binet Test, Form L, the boy made a mental age of 3 years, thus giving him an Intelligence Quotient of 44. He was also tested on the Seguin Form Board and the Manikin Test and the results were similar to those made on the Stanford Binet Test. On the Vineland Social Maturity Scale, the boy obtained a Social Quotient of 74.

During his entire stay at the Home the boy was very tractable, but all his reactions were characteristic of a markedly intellectually retarded child. However, he seemed more alert than his brother (Case 1). He was able to carry out his routine duties fairly satisfactorily, although he also needed constant supervision. His memory was poor both for recent and past events. His speech was more intelligible than his brother, although he was able to speak only a few words very plainly. There was no indication of any psychotic behavior.

**Discussion.** The clinical and laboratory findings as well as the data from the social history permit the diagnosis of Gargoylism in these 2 cases.

Both cases showed the characteristic skeletal deformities, marked enlargement of the spleen and liver, umbilical hernia and feeble-mindedness.

One of the cardinal symptoms, namely, corneal clouding was absent. However, this is not of great significance: Cordes and Hogan<sup>1</sup> have pointed out that corneal clouding is absent in about 25% of the cases. Even when present, it may be very difficult to detect and hence may be overlooked. This is especially true, as in our cases, where slit-lamp examination was not feasible.

Of the laboratory tests, the most significant were those concerned with liver function, all of which gave abnormal values, showing definite liver damage. All the other laboratory examinations including the electro-encephalogram and the electrocardiograms were of interest only from the standpoint of negative findings.

The roentgenograms of the bones showed marked developmental retardation as well as structural changes. The skulls were enlarged but there was nothing characteristic noted about the sella turcica. The shortening and thickening of the bones of the hands as well as the incurving of the fingers were striking.

It has been definitely established that Gargoylism is of congenital origin and familial in nature. It affects both sexes. Consanguinity apparently plays no rôle. Neither are the parents of such children

mentally defective. In our cases, there was no consanguinity, neither were the parents mentally retarded.

From the pathologic-anatomic standpoint, Gargoylism has been placed among the lipoidoses, abnormal storage of lipid-like substances being found in various tissues, principally in the reticulo-endothelial cells, the brain, cornea, pituitary gland, liver, spleen and lymph nodes. The deposits of lipid material appear in the form of fine, curved rod-like granules whose exact chemical nature is as yet undetermined but are said to be probably a complex compound of lipid and protein.

The bony changes, however, cannot be explained on the basis of the lipid disturbance, as Washington<sup>7</sup> has proven. According to him, the enlargement of the skull in these patients is due to a defect in the mesodermal anlage of the top of the skull, while the anomalies in the vertebræ result from stresses on the bodies of the vertebræ whose cartilages have failed to develop. The elongation of the sella turcica as seen in the roentgenograms as well as the depression of the bridge of nose and the widening of the orbits are caused by abnormal development of the sphenoid bone. The disturbance in the epiphyseal growth resulting in dwarfism is probably due to the pituitary involvement. The restricted motion in the finger, wrist and elbow joints is apparently due to poor formation of the articular surfaces.

All the other symptoms, such as the mental retardation, the sexual infantilism, the enlargement of the liver and spleen and the ocular signs are probably caused by the infiltration of the lipid-like substance into these organs.

In regard to the symptomatology, most reports agree that at birth there are usually no abnormalities present. In a few cases, enlargement of the head and some cloudiness of the cornea have been noted at birth. However, in the majority of cases, the first symptoms usually appear between the 1st and 2d year of life when a failure in the normal development of these children is noted. The enlargement of the head and the dorsolumbar kyphosis are usually noted at this time. Growth soon seems to stop and normal physical and mental development ceases altogether, and the child begins to assume the characteristic features which give this condition its name.

This condition must be differentiated from Morquio's disease.<sup>6</sup> The principal differential diagnostic signs are that in the latter condition, there is no limitation of motion of the joints of the body, and the children are not feeble-minded.

**Summary.** Two cases of Gargoylism, occurring in brothers, are reported; and the etiology, clinical and laboratory findings, pathology and differential diagnosis are discussed.

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## SURGICAL REMOVAL OF ADRENAL ADENOMA WITH RELIEF OF CUSHING SYNDROME

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IN 1941, a 46 year old man entered the Beth Israel Hospital presenting the classical Cushing syndrome. The presence of an adrenal cortical neoplasm was suspected from the pyelographic studies and later confirmed by perirenal insufflation and laminography.<sup>16</sup>

The excretion of androgens in the urine was considerably elevated, whereas the estrogens were within normal limits, suggesting, according to Frank,<sup>8,9</sup> the probability of a benign tumor.

At operation a large encapsulated adenoma of the right adrenal gland was excised and after a stormy postoperative course the patient made a complete recovery. Sixteen months after the operation, he felt well and appeared normal, having lost all the stigmata of his former disease. The postoperative course presented several typical and difficult problems.

**Case Report.** Beth Israel Hospital No. 137160. A 46 year old man entered the hospital on November 18, 1941, complaining of dizziness and irritability. He was well until 1½ years ago when he noticed blurring of vision of his left eye. At another hospital a diagnosis of diabetes and hypertension was made. On the advice of physicians he submitted to the extraction of his teeth and later to tonsillectomy and vaccine injections. The dizzy spells continued, however, and were associated with headaches, poor memory and irritability. There was no nausea or vomiting. For the past 6 months the patient was sexually impotent.

The patient's general appearance suggested Cushing syndrome (Fig. 1) with marked adiposity of the face, neck and abdomen. The face and neck were dusky red and there were purplish striae on the abdomen. The testes were small and flabby and the extremities were thin.

The blood pressure was 190 systolic and 118 diastolic. In the next 2 weeks the systolic pressure varied between 190 and 162, and the diastolic between 118 and 106.

Urine examination showed glucose 4+ and albumin, heavy trace. On November 26 and on December 8 the urine again showed glucose 4+, but on December 19 and January 2, it was negative.

Blood glucose on November 19 was 168 mg. and on December 4, 244 mg. Blood non-protein nitrogen on November 19 was 35 mg. Red blood cells were 4,930,000, hemoglobin 100, white blood cells 7600, polymorphonuclears 70%, mononuclears 30%. A Roentgen ray of the skull showed diffuse osteoporosis.

Excretory urography revealed a large circular shadow in the right suprarenal region with downward displacement of the right kidney. The left kidney was normal and the function on both sides was good.

On December 27, 500 cc. of filtered air was injected into the right perirenal space and the simple film and laminogram revealed a large tumor in the right suprarenal region (Figs. 2 and 3).

Androgen determination (17-ketosteroids) of the urine colorimetrically on November 22 showed 92.75 mg. excreted in 24 hours, a marked elevation. Estrogen excretion on November 22 was 110 international units calculated as estrone, or 11  $\mu$ g. for 24 hours. This is within the normal range.

On a salt-poor diet the patient lost 10 pounds and the blood pressure fell to 152 systolic, 104 diastolic. On November 30, December 1 and 2, 10 cc. of desoxycorticosterone (cortate) was administered.



FIG. 1.—Preoperative photograph showing adiposity of face, neck and abdomen.

*Operation.* On December 3, 1941, the right kidney and adrenal were exposed through a lumbar incision (S. F. W.). The kidney appeared normal, but directly above it there was an encapsulated tumor mass about the size of an orange. Following resection of the 11th and 12th ribs, the tumor was freed from the posterior parietes and the diaphragm, and delivered into the wound attached only by a narrow vascular pedicle. The pedicle was ligated, doubly clamped and cut, and then again ligated. The kidney was replaced and the wound closed in layers, 1 gauze packing and 1 rubber dam drain coming out of the posterior angle.

*Pathologic Report* (Drs. A. Plaut and H. Brody). Gross: Irregularly ovoid, moderately soft, tumor mass, 12 x 9.5 x 4.5 cm., weighing 284 gm. (Fig. 4). Except at one pole the tumor appears encapsulated. At this pole it is torn and its original state can no longer be judged. Stretched over one surface, over an area about 4 cm. in length and less than 1 cm. in width, a small fold of normal-appearing suprarenal tissue can be recognized. Through the capsule the tumor appears irregularly lobulated; it varies from yellow-brown to brown; large blood-vessels course beneath the capsule.



FIG. 2.—Simple film following perirenal insufflation, showing large tumor above right kidney. Oblique view.

On incision, corresponding to the external appearance, a small piece of suprarenal cortex is applied to the tumor. The width of this piece is slightly under 1 cm. On its cut surface two almost paper-thin, closely applied, folded cortical layers can be made out. These layers are very pale yellow, apparently very poor in lipoid. No medullary zone can be recognized. At some levels this portion of adrenal tissue is separated from the underlying tumor by a gray stripe less than 0.5 mm. in thickness, suggestive of connective tissue. It does not have the characteristics of normal medulla.

The tumor has a distinctly lobulated appearance. There is marked variation in the appearance of the different lobules. Perhaps one-third to one-half of the tumor is necrotic, consisting of soft salmon-pink and bloody material. Adjacent to these necrotic portions numerous small thrombosed blood-vessels can be seen. The other nodules for the most part are a dull, light purple-

brown, but yellow areas are scattered about. Some of these have the color which is usually associated with the lipoid in adrenal cortex. Numerous cavities are present, some of which are also lined with similar bright yellow tissue. Some of the areas are peculiarly stippled with yellow and light brown, some areas are more opaque, others more translucent. There are occasional chalky yellow areas, presumably of necrosis. *Sections* were placed in various fixatives, the majority of the tissue being used for chemical analysis.

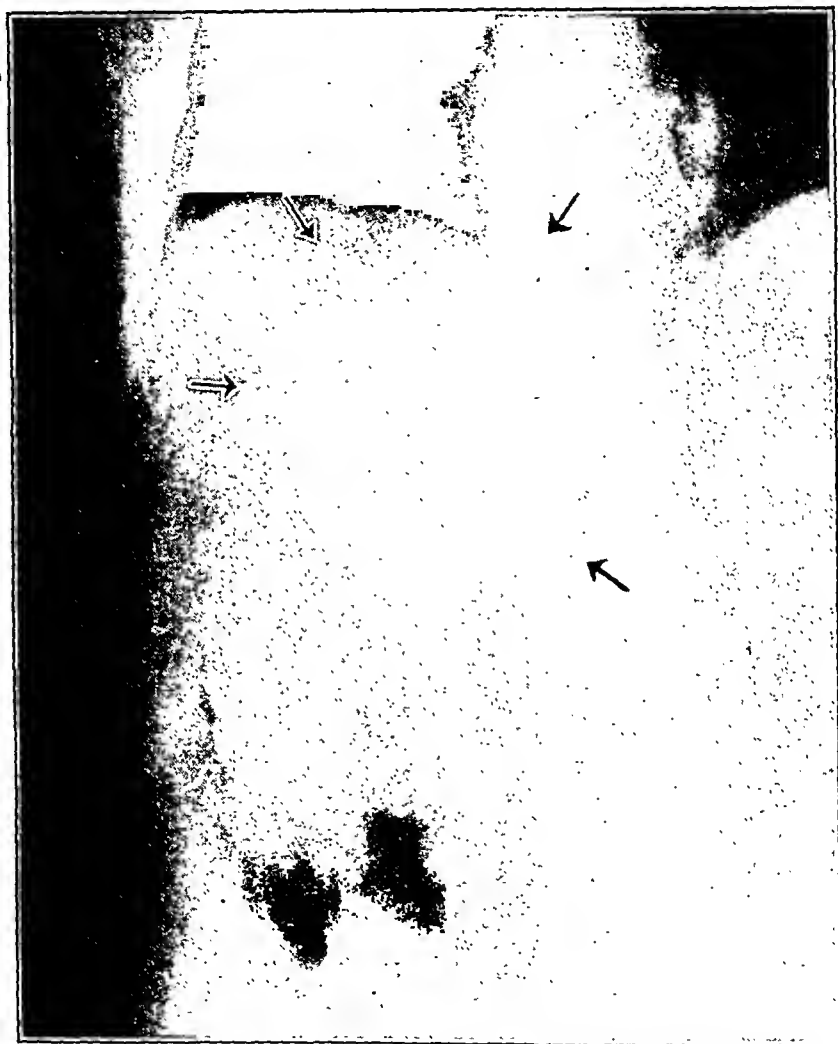


FIG. 3.—Laminograph delineating tumor of right adrenal gland.

*Microscopic.* Corresponding to the gross picture, a small cap of suprarenal tissue rides on the large tumor. At a few points a suggestion of zona glomerulosa can be made out. The fasciculata is greatly distorted and its cells are large and fat-laden. The fat is anisotropic. No medullary tissue is found. The central veins are thick-walled. The suprarenal tissue is separated from the tumor by a connective tissue capsule and the suprarenal is stretched and thinned out along the capsule.

The tumor is composed of coarse strands several cells in thickness, separated by a delicate connective tissue network (Fig. 5). In some places the strands broaden to form large sheets of cells. The cells are large and polygonal. The

cytoplasm is for the most part finely granular, although most cells show hyaline eosinophilic areas. The nuclei tend to be centrally located, are fairly regular, though large and occasionally rather bizarrely shaped nuclei are found. The cells contain only occasional sudanophilic granules which are not anisotropic. However, in the areas which were grossly bright yellow, much anisotropic lipoid is present. The tumor contains numerous large vascular spaces with thin walls about which the tumor cells are oriented at right angles.



FIG. 4.—Encapsulated adrenal adenoma. Operative specimen.



FIG. 5.—Section of adrenal adenoma,  $\times 160$ .

There are fairly extensive areas of hemorrhage, necrosis and inflammation. *Chemical Analysis* (Drs. M. S. Biskind and M. C. Shelesnyak). Part of the tumor was ground and 2 aliquot samples were extracted with carbon tetrachloride, one in the vacuum extractor<sup>14</sup> and the other with the Soxhlet extractor. The phenolic (estrogen) fraction was separated and androgens (17-ketosteroids) were determined colorimetrically. Identical results were

obtained with both extracts: 1.14 mg. androgenic 17-ketosteroids per gram of tissue, or a total of 323.76 mg. for the tumor.

*Postoperative Course.* The postoperative treatment was directed toward the prevention of adrenal cortical insufficiency. The patient was prepared before operation with the daily administration of 40 mg. of methyl testosterone for 4 days and 10 mg. of cortate daily for 3 days. An hour before operation 1 cc. of cortical adrenal extract (Upjohn) was given intramuscularly.

During the operation a transfusion of 500 cc. of whole blood was given, followed by a continuous intravenous infusion of 0.5% sodium citrate, 1% sodium chloride, and 10% glucose. Frequent blood pressure readings served as a guide for the administration of adrenal cortical extract.

The patient's condition was excellent immediately after operation. The blood pressure was: systolic 160, diastolic 100. The blood pressure level dropped slowly during the day, but the systolic pressure remained above 100 until noon of the next day (December 4). During the first 24 hours after operation, 19 cc. of adrenal cortical extract and 10 mg. of cortate were given in divided doses. In addition, 50 mg. of testosterone were injected.

During the next 24 hours, from noon, December 4, until noon, December 5, the systolic pressure fluctuated between 78 and 108, and the temperature between 102.8° and 104.6° F. The pulse was rapid and irregular, the abdomen distended, and the patient was weak. During these 24 hours, 40 cc. of adrenal cortical extract was administered in divided doses. Normal saline and 5% glucose were given intravenously. Vitamins and calcium gluconate were administered. The urinary output was satisfactory.

During the next 3 days (December 5 to 8) the systolic pressure varied between 120 and 132. Sixty cc. of adrenal cortical extract, 10 mg. of desoxycorticosterone, and 50 mg. of testosterone were given. The patient's condition was good, the temperature remaining at lower levels until December 7, when there was a rise to 103.8° F. with a chill.

On the 5th postoperative day (December 8) the wound was found to be infected. The granulations were pale and in some places necrotic. The wound was opened widely and irrigated with Dakin's solution.

Urinary output was satisfactory until December 10, when the volume of urine was 360 cc. From December 8 to December 10, 35 cc. of adrenal cortical extract was administered, in addition to a large amount of saline solution. The systolic pressure fluctuated between 110 and 136.

On December 10 the patient was very ill, the pulse and respiration were rapid, and the temperature around 103° F. The abdomen was distended and the wound infection was unabated. The extremities became edematous. The blood sodium was 335.8 mg. per 100 cc. and the blood potassium 15.8 mg. per 100 cc.

During the next 24 hours the urinary output was 600 cc. and the edema increased. Ten cc. of adrenal cortical extract had been given but this now was discontinued along with the sodium chloride. Sulfadiazine was administered to combat the resistant wound infection and suspected bronchopneumonia.

On December 12 the wound infection had extended on to the anterior abdominal wall as a brawny cellulitis. This area was widely incised with the evacuation of thin foul pus. The tissues were edematous and in places necrotic. A transfusion of 350 cc. of whole blood was followed by an infusion of 5% glucose in distilled water. The high fever persisted and the patient's condition became steadily worse.

On December 13, the cellulitis had spread and there was visible generalized edema. The urinary output was 460 cc. in 24 hours. At 10.30 A.M., 1.1 cc. of mercupurin and 7.5 gr. of aminophyllin were injected and in the following 48 hours 7660 cc. of urine were voided. That afternoon at the suggestion of Dr. Arthur Fishberg the daily administration of 3 gm. of potassium chloride was begun. Sodium chloride and adrenal extracts were prohibited.

On December 14, the patient appeared better and the systolic pressure remained between 154 and 160. A transfusion of 250 cc. of whole blood was given,



On December 15, the patient's condition had greatly improved, the edema was diminished and the temperature had fallen to 102° F. On the next day potassium and sulfadiazine were discontinued.

For the following 2 weeks the patient improved decidedly and the blood pressure was maintained at levels above 130 systolic and 76 diastolic without adrenal cortical extract or desoxycorticosterone. Androgen determination of the urine on December 22 showed 15.24 mg. for 24 hours, which is within the normal range.

On December 26, the patient began to complain of weakness, persistent nausea and occasional vomiting. The blood sodium was 297.9 mg. per 100 cc. and the blood potassium 22.3 mg. per 100 cc. On December 27 and 28, 5 mg. of desoxycorticosterone were given, and on each of the next 4 days, an average of 5 cc. of adrenal cortical extract. Following ingestion of 1.5 gm. of potassium chloride, the systolic pressure fell to 100.

Ten cc. of adrenal cortical extract were then injected daily until January 9, when the daily dose was increased to 15 cc. On January 2 the urine was negative for glucose and on January 3, 350 cc. of whole blood and an infusion of glucose and saline were given. Despite the nausea and vomiting, the patient improved and sat out of bed on January 9. The temperature then rose to 101.8° F.

On January 12 a slightly tender rounded mass about 6 cm. in diameter was felt in the epigastrium. The mass grew larger and more tender, but, when aspirated on January 14, no pus was obtained. Culture showed *Staph. aureus*.

At operation (S. F. W.) on January 16, a circular fatty tumor 7.5 cm. in diameter was found between the peritoneum and deep rectus fascia. On opening the peritoneum, the mass was seen extending along the falciform ligament with smaller infiltrated fatty nodules in the omentum. The mass was largely but not completely excised and the pathologic report (Dr. A. Plaut) showed inflamed fat tissue.

The patient gradually improved, the wound healing by primary union. The systolic blood pressure fluctuated between 120 and 140 and on January 18, adrenal cortical extract was finally discontinued. Despite a mild phlebitis of the left leg on January 25, the patient was out of bed on February 4. He was much improved and had lost considerable weight. The temperature was normal and the blood pressure was 120 systolic and 92 diastolic. The testicles were firmer and the patient left the hospital on February 8.

*Follow-up.* On April 8, 1942, the patient was weak but felt well. His physical appearance had changed remarkably. He was thin and looked younger. The red coarseness of the facial skin had disappeared. The blood pressure was 140 systolic, 100 diastolic. The testes were larger and firmer and sexual potency had returned.

On May 25, the patient felt much stronger.

On October 16, the blood pressure was 130 systolic, 90 diastolic. A 24-hour specimen of urine contained 20 mg. of androsterone (non-phenolic 17-ketosteroids).

On December 28, the blood pressure was 120 systolic, and 80 diastolic. Both incisions were firmly healed. The testes were normal and sexual potency continued. The patient felt well but had gained weight by overeating.

On March 13, 1943, the patient said that he was perfectly well, but tired after heavy exertion. The systolic pressure was 128 and the diastolic 85.

**Comment.** Cushing<sup>6</sup> in 1932 described the now well-known syndrome characterized by plethoric adiposity of the face, neck and trunk, purplish striae, acrocyanosis, polycythemia and vascular hypertension. This disorder was frequently accompanied by hyperglycemia and osteoporosis, and in women by amenorrhea and hypertrichosis. The finding of a basophilic adenoma of the pituitary at autopsy in 3 of his cases led Cushing to believe that this lesion was the primary cause of the clinical picture. This belief is not commonly accepted.

The association of some of these symptoms with neoplasm of the adrenal gland has been known since 1756 when William Cooke<sup>5</sup> reported his observations on a 7 year old girl who suffered from enormous adiposity and a copious growth of facial and genital hair. Since then many observers, including Apert,<sup>1</sup> Young,<sup>17</sup> Oppenheimer and Silver<sup>13</sup> and others<sup>4,10,15</sup> have reported examples of a clinical picture similar to, or practically identical with Cushing syndrome caused by adrenal cortical neoplasm. Arrhenoblastomata of the ovary and thymic tumors have also been reported with similar clinical features.

Although the first successful operation for adrenal tumor was done in 1889, it is only in the last decade that Broster and Vines,<sup>3</sup> Walters, Wilder and Kepler,<sup>15</sup> Young,<sup>17</sup> Cahill<sup>4</sup> and others<sup>10,12</sup> have reported any considerable number. Most of these occurred in women.

Adrenal operations for classical Cushing syndrome are quite uncommon, and survival following the extirpation of an adrenal neoplasm in these cases is indeed rare. Walters, Wilder and Kepler<sup>15</sup> reported great improvement in a 9 year old girl 6 months after operation. The remainder of their cases, as well as those of Cahill, Oppenheimer and Silver, and others, either died or suffered from adrenal carcinoma. Several patients succumbed shortly after operation in acute adrenal insufficiency. In some of these autopsy revealed atrophy or absence of the contralateral adrenal gland.<sup>2,7</sup> Others progressed favorably for several days but then gradually failed, becoming weaker, and finally succumbing to an intercurrent infection.<sup>13</sup> Such patients apparently suffered from a subacute adrenal insufficiency despite vigorous replacement therapy consisting of adrenal gland extract, desoxycorticosterone, and saline infusions. Atrophy of the contralateral adrenal in these cases is in all likelihood the result of prolonged hypersecretion of adrenal hormones by the neoplasm.

In cases of carcinoma, on the other hand, the danger of postoperative adrenal insufficiency is obviously much reduced by the continued secretion of hormones by neoplastic residua and metastases.

**Summary.** A 46 year old man presenting a classical Cushing syndrome was found to be suffering from a large adenoma of the right adrenal gland. He was completely relieved of his symptoms following extirpation of the tumor and has remained well for over 16 months after the operation.

Laminography and perirenal insufflation proved valuable in delineating the neoplasm.

The urinary androgens (17-ketosteroids) were greatly elevated. The normal excretion of urinary estrogens suggested the likelihood of a benign neoplasm.

The postoperative treatment was directed toward the prevention of adrenal cortical insufficiency. Frequent blood pressure readings served as an important guide for the administration of adrenal cortical extract, desoxycorticosterone, and saline infusions. On several occasions when the systolic blood pressure fell below 100, adrenal cortical extract (Upjohn) was administered intravenously with gratifying results. The efficacy of intravenous adrenal cortical extract precludes the necessity for prolonged preoperative preparation.

The danger of overtreatment with adrenal cortical extract, desoxycorticosterone, and saline infusion was vividly illustrated from the 7th to the 12th postoperative day. Oliguria and severe anasarca were successfully combated by the discontinuance of adrenal cortical extract, desoxycorticosterone, and sodium chloride, and by the prompt administration of mercupurin, potassium chloride, and 5% glucose in distilled water.

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## HEART DISEASE IN SELECTIVE SERVICE EXAMINEES

## A STUDY OF 20,000 EXAMINEES IN THE PACIFIC NORTHWEST\*

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HEART disease occupies a major place among the nation's health problems, and is at present responsible for an appreciable percentage of rejections from military service. This study was undertaken to determine the types and frequency of heart disease among 20,000 consecutive Selective Service examinees appearing before the Army Examining Board at this station. No such survey has appeared thus far.

Rowntree, McGill and Folk<sup>6</sup> in a statistical survey of the physical and mental causes for rejection in 18 to 36 year old examinees, reported cardiovascular defects to be responsible for an estimated 10.6% of

\* At the time this study was conducted the Station was situated in Tacoma, Wash., functioning as the U. S. Army Examining and Induction Station.

total rejections. More recently, in a similar survey in Tennessee, Fenn and his associates<sup>3</sup> reported rejections for cardiovascular defects as 4.4% of the men examined. However, it should be noted that these figures include, in addition to heart disease, the various other cardiovascular causes for rejection such as hypertension without cardiac disease, peripheral vascular disease, persistent tachycardia, and (in Rowntree, McGill and Folk's study) varicose veins. McKinlay<sup>4</sup> reported heart disease as responsible for 10.5% of 209 Selective Service examinees rejected by local board examiners in Minnesota for defects within the field of internal medicine.

Our study is based on the examinations of 20,000 consecutive Selective Service examinees, ranging in age from 20 to 45 years, and representing a cross-section male population of corresponding age in the western half of the state of Washington.\* These include urban and rural inhabitants: Professional men, white collar workers, skilled and unskilled laborers, and farmers. The vast majority is of the white race. A small percentage (less than 3%) include the Indian, negro, Chinese, and Filipino races, of which the Indian is the most common.

Diagnosis was based upon the criteria adopted by the American Heart Association,<sup>5</sup> and recommended by the War Department,<sup>7</sup> and was established by history, physical examination, exercise tolerance, stereoroentgenograms,† and when indicated, electrocardiograms.

TABLE 1.—TYPES AND FREQUENCY OF HEART DISEASE ENCOUNTERED IN 20,000 EXAMINEES

Type of heart disease	No. of cases	Total cardiac rejections (%)	Rejections per 1000 examinees
Rheumatic . . . . .	183	63.5	9.15
Congenital . . . . .	35	12.2	1.75
Arteriosclerotic . . . . .	9	3.1	0.45
Hypertensive . . . . .	6	2.1	0.30
Hyperthyroid . . . . .	3	1.0	0.15
Effort syndrome (neurocirculatory asthenia)	3	1.0	0.15
Paroxysmal tachycardia . . . . .	2	0.7	0.10
Chronic constrictive pericarditis . . . . .	1	0.3	0.05
Organic disease of unknown etiology . . . . .	46	16.0	2.30
Total . . . . .	288	99.9	14.40

TABLE 2.—REJECTIONS FOR HEART DISEASE AS COMPARED TO REJECTIONS FOR ALL PHYSICAL AND MENTAL DEFECTS IN THE 20,000 MEN EXAMINED

Cause for rejection	No. of rejections	Total examinees (%)	Relative‡ percentage
Heart disease . . . . .	288	1.44	6.0
All physical and mental defects . . . . .	4820	24.10	100.0

**Incidence and Types of Heart Disease.** Heart disease was observed in 288 of the 20,000 men examined, an incidence of 1.44%, or 14.4 cardiac rejections per 1000 men examined. Rejections for cardiac

\* All Army induction examinations for the western half of the state of Washington are conducted at this Station.

† Stereoroentgenograms were 4 x 5 inches in size, and taken at a target-film distance of 42 inches. Proper consideration was given this factor.

‡ Percentage cardiac rejections as compared to total physical and mental rejections.

disease constituted 6% of total rejections for physical and mental defects (Table 2). Table 1 summarizes the various types and frequency of cardiac disease encountered. Rheumatic heart disease was the most common, occurring in 183 examinees, or 63.5% of total rejections for heart disease and 9.15 men per 1000 examined. Congenital heart disease was observed in 35 instances, or 12.2% of cardiac rejections and 1.75 men per 1000 examinees. Arteriosclerotic heart disease was observed in 9 instances or 3.1% of heart disease encountered, and 0.45 men per 1000 examined; in this group were 3 cases of myocardial infarction (all less than 1 year old), and 1 man exhibiting a well-defined anginal syndrome. Six cases (2.1% of rejections for heart disease; 0.3 men per 1000 examined) of hypertensive heart disease were present; these do not include examinees rejected for hypertension without cardiac disease. Three cases (1% of cardiac rejections; 0.15 men per 1000 examined) of hyperthyroid heart disease were noted and were confirmed by high basal metabolic rates. Unequivocal effort syndrome (neurocirculatory asthenia) was encountered in only 3 instances (1% of cardiac rejections; 0.15 per 1000 men) in our series. Two examinees (0.7% of cardiac rejections; 0.1 men per 1000) were rejected for paroxysmal tachycardia.\* One case (0.3% of cardiac rejections; 0.05 men per 1000 examined) of chronic constrictive pericarditis was noted. Forty-six cases (16% of cardiac rejections; 2.3 men per 1000 examined) of organic heart disease of undetermined etiology were encountered in our series.

Table 3 represents the relative frequency of the various types of valvular involvement found in those men rejected for rheumatic heart disease. No examinee was rejected merely on the basis of a history

TABLE 3.—VALVULAR DEFECTS IN EXAMINEES REJECTED FOR RHEUMATIC HEART DISEASE

Valvular defect	No. of cases	Per cent
<i>Mitral</i> . . . . .	152	83.1
Insufficiency . . . . .	92	50.3
Stenosis . . . . .	22	12.0
Stenosis and insufficiency . . . . .	38	20.8
<i>Aortic</i> . . . . .	8	4.4
Insufficiency . . . . .	4	2.2
Stenosis . . . . .	1	0.5
Stenosis and insufficiency . . . . .	3	1.6
<i>Combined mitral and aortic</i> . . . . .	23	12.5
Total . . . . .	183	100.0

of rheumatic fever. The mitral valve was involved alone in 152 (83.1%) of the 183 cases, mitral insufficiency occurring in 92 men (50.3%), mitral stenosis in 22 (12%), and mitral stenosis and insufficiency in 38 (20.8%). The aortic valve was involved alone in only 8 examinees (4.4%); in these, aortic insufficiency was noted in 4 men (2.2%), aortic stenosis in 1 examinee (0.5%), and aortic stenosis and insufficiency in 3 examinees (1.6%). Combined mitral and aortic

\* Examinees subject to attacks of paroxysmal tachycardia are not acceptable for military service (War Dept., Mobiliz. Reg. No. 1-9, Sect. XIV, Par. 64d, March 15, 1942).

lesions were present in 23 men (12.5%). In the last group, although specific diagnoses of the character of valvular involvement were included in our examination records, we are omitting these in this report because of the significant percentage of error undoubtedly associated with such attempted accuracy.

The predominant congenital anomalies observed in the men rejected for congenital heart disease are summarized in Table 4. Interventricular septal defect was the most frequent anomaly noted, occurring in

TABLE 4.—PREDOMINANT CARDIAC DEFECTS IN EXAMINEES REJECTED FOR CONGENITAL HEART DISEASE

Predominant cardiac defect	No. of cases
Interventricular septal defect . . . . .	11
Patent ductus arteriosus . . . . .	5
Interventricular septal defect with congenital A-V block . . . . .	1
Interauricular septal defect . . . . .	1
Coarctation of the aorta . . . . .	1
Pulmonary stenosis . . . . .	1
Undetermined types . . . . .	15
Total . . . . .	35

11 examinees. Patent ductus arteriosus was observed in 5 men. One instance of interventricular septal defect and congenital complete auriculoventricular block was encountered in a 39 year old man. One case each of interauricular septal defect, coarctation of the aorta and pulmonary stenosis were present, the last associated with moderate cyanosis and marked clubbing of the fingers. There were 15 cases of congenital heart disease of undetermined type.

Early to moderate congestive heart failure was observed in 5 instances, occurring in 2 examinees with rheumatic heart disease and mitral stenosis (1 of which also exhibited auricular fibrillation), in 1 examinee with congenital heart disease, in 1 with hypertensive heart disease, and in 1 with organic heart disease of unknown etiology. Two cases of auricular fibrillation were encountered, both occurring in examinees over the age of 40, with rheumatic heart disease and mitral stenosis.

TABLE 5.—COMPARISON BETWEEN INCIDENCE OF HEART DISEASE AND FUNCTIONAL MURMURS

	No. of cases	Total examinees (%)
Heart disease . . . . .	288	1.44
Functional murmurs . . . . .	297	1.48

It might be of interest to comment briefly on several non-disqualifying cardiac conditions encountered in the 20,000 men examined. Among these were 2 instances of congenital dextrocardia, and 1 man with a previous bullet wound of the heart, the bullet having been surgically excised, with subsequent cardiac function remaining unimpaired. Functional murmurs were noted in 297 (1.48%) of the 20,000 men examined, an incidence slightly higher than that observed for all forms of cardiac disease combined (Table 5). The occurrence of premature systoles was relatively infrequent, and may be due, as sug-

gested by White,<sup>8</sup> to the cardiac acceleration resulting from the nervous tension and excitement associated with the induction examination. The majority of the examinees exhibited heart rates between 85 and 100.

**Comment.** It is intended that this report be as brief as possible. However, several recent studies warrant mention despite the fact that the ages represented are not strictly comparable. Cole,<sup>1</sup> in a survey of the incidence of heart disease among 28,139 students at the University of Wisconsin, observed organic heart disease in 1.02% of the students, with a sex relationship of 1.7 females to 1 male. Cuykendall<sup>2</sup> observed an incidence of 1.1 (54 cases) of 4991 male students at Cornell University. Rheumatic heart disease comprised 0.8%, and congenital heart disease 0.26% of this number; in addition 1 case of subacute bacterial endocarditis was encountered.

The absence of syphilitic heart disease in our series warrants comment. It is apparently the result of 3 important factors: (1) examinees with positive serologic reactions for syphilis were disqualified from appearing before the Army Examining Board; (2) the low incidence of negro examinees at this station; and (3) the relatively small percentage of examinees over the age of 40 (approximately 9%). In view of the last, it is probable that the incidence of syphilitic heart disease would have been low regardless of the preceding two factors, and consequently would not have significantly influenced the number of rejections for heart disease.

**Summary.** 1. A study of 20,000 consecutive Selective Service examinees, representing a cross-section of male population 20 to 45 years of age in the Pacific Northwest, revealed the existence of heart disease in 288 men, an incidence of 1.44%. This figure constituted 6% of rejections for all physical and mental defects.

2. Rheumatic heart disease was observed in 183 examinees, or 63.5% of cardiac rejections and 9.15 men per 1000 examined. Congenital heart disease followed in frequency, occurring in 35 men (12.2% of rejections for heart disease, or 1.75 men per 1000 examined). There were 9 instances of arteriosclerotic heart disease, 6 examinees with hypertensive heart disease, 3 cases each of hyperthyroid heart disease and effort syndrome (neurocirculatory asthenia), 2 instances of paroxysmal tachycardia, and 1 case of chronic constrictive pericarditis.

3. There were 46 cases of organic heart disease of unknown etiology, or 16% of total cardiac rejections and 2.3 men per 1000 examined.

4. In the 183 examinees rejected for rheumatic heart disease the mitral valve was involved alone in 152 cases (83.1%), the aortic valve was involved alone in 8 examinees (4.4%), and combined mitral and aortic valve defects were observed in 23 cases (12.5%).

5. Functional murmurs were noted in 297 examinees, an incidence of 1.48% of the total number of men examined. This figure represents an incidence slightly higher than that observed for all forms of cardiac disease combined.

6. The figure of 288 men rejected for heart disease (1.44% of 20,000 examinees) is of interest in comparison with the 4820 rejected for all physical and mental defects (24.1% of examinees). (See Table 2.)

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## A CLINICAL AND BIOCHEMICAL STUDY OF COW'S MILK AND HONEY AS AN ESSENTIALLY EXCLUSIVE DIET FOR ADULT HUMANS\*

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THE nutritional deficiencies of cow's milk for adults are at present recognized as consisting largely of inadequate concentrations of iron, copper and manganese and frequently iodine, and a low caloric value in proportion to its bulk. The ascorbic acid content of most pasteurized milk is also inadequate. Although the thiamine content is actually quite low because of the high water content, it is adequate in relation to its caloric value. According to Melnick,<sup>25</sup> the thiamine requirement of adult humans is 350 micrograms per 1000 Calories. Whole cow's milk having 3.5% fat contains, on the average, 750 micrograms of thiamine per 1000 Calories. The surprisingly low nicotinic acid content of cow's milk has not been yet reconciled with its established antipellagra value. The importance for adults of the normally low vitamin D has not been determined.

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There have been very few experimental studies in which milk was the major article of diet for adults for a considerable period of time. Neff<sup>31</sup> described an experiment in which 9 men limited their diet for 1 month to plain milk or gelatinized milk, with a supplement of 1 or 2 oranges daily. All the subjects continued their regular laboratory work without interruption and apparently remained in excellent health. Irwin,<sup>21</sup> describing the beneficial effects of milk as a dietary adjunct, states, that "For 3 months one winter several men students lived on raw milk supplemented with the missing minerals and with orange juice. They drank as much as they wanted—usually  $3\frac{1}{2}$  to  $4\frac{1}{2}$  quarts a day. They maintained their weight, in fact some of the boys gained, and they all felt well and satisfied." This experiment apparently has not been published in detail.

Experimental animals have been employed for studies on the effect of extended feeding of liquid cow's milk fortified with minerals or calorogenic substances. Daniels and Hutton<sup>11</sup> reported the successful rearing of 5 generations of rats on boiled milk after addition of only iron, iodine, aluminum, fluorine, manganese and silicon salts. Daniels and Everson<sup>10</sup> found that a boiled milk-iron-copper diet sufficed for 4 generations but that manganese was necessary for a 5th generation. Kemmerer *et al.*<sup>23</sup> secured as good growth and development of rats and pigs on cow's milk supplemented with iron, copper and manganese as with a stock ration. . Anderson *et al.*<sup>1</sup> found that a supplement of iron, copper, manganese and cod-liver oil to raw cow's milk made it adequate for 2 generations of dogs, and maintained male dogs in normal health for 3 years.

Van Donk and co-workers<sup>49</sup> found that the addition of sucrose to mineralized cow's milk improved its value for growth, ovulation and reproduction of rats. The comparative chemical composition of milk and honey (average analyses) shows that dark honey is 4.6 times richer in caloric value, 5.3 times richer in iron, 100 times richer in manganese and 7.3 times richer in copper than cow's milk which would seem to make it an ideal supplement so far as its energy and mineral deficiencies are concerned. Light honey is poorer in these mineral constituents (Table 1). Haydak, Palmer and Tanquary<sup>17</sup> have shown that a cow's milk-dark honey diet will both cure and prevent nutritional anemia in rats.

Haydak<sup>16</sup> subsisted for 3 months on a cow's milk-honey mixture consisting of 100 gm. of honey per quart of milk. He found that his ability to work was normal and that no sluggish or tired feeling developed. The clinical observations were limited but showed no loss of weight, normal bowel movements, absence of proteinuria and glycosuria and slight rise in hemoglobin content of the blood. However, toward the end of the experiment, certain symptoms suggestive of vitamin C deficiency were noticed, consisting of fresh blood in the feces, dryness of the skin, a hyperemic area on the gums, numbness and ulceration of the tongue and small reddish papulæ on the forehead and face. All these symptoms were promptly cured by orange juice.

The present study was prompted by a desire to conduct a more

extensive investigation of the type carried on by Haydak, involving a larger number of subjects, a much more complete biochemical analysis of the blood and urine as well as a more comprehensive clinical and dental study.

TABLE 1.—COMPOSITION OF MILK AND HONEY. AMOUNTS PER 100 CC. OR GM.

	Milk	Honey	
		Light	Dark
Calories . . . . .	69	320	320
Protein, gm. . . . .	3.5		
Ca, mg. . . . .	120		
P, mg. . . . .	93		
Cu, mg. . . . .	.015	.030*	.11*
Fe, mg. . . . .	24	.24*	1.28*
Mg, mg. . . . .	12		
Mn, mg. . . . .	.004	.03†	.41†
Thiamine, µg. . . . .	50	5.5	5.5
Riboflavin, µg. . . . .	190	61	61
Ascorbic acid, mg. . . . .	2	2.4	2.4
Nicotinic acid, mg. . . . .	.21	.36	.36
Pantothenic acid, µg. . . . .	.40	.10	.10

\* Haydak, Palmer, Tanquary.<sup>17</sup>

† Schuette and Remy.<sup>39</sup>

**Diet and Methods.** Five healthy individuals (4 males and 1 female) varying in age from 22 to 44 years were put on the diet consisting of a mixture of 1 quart of pasteurized milk with 100 gm. of light honey. The University creamery supplied the milk. A mixed honey (from sweet and white clover) from the University apiary was used throughout the experiment. The diet was carefully prepared daily, bottled and distributed to all the experimental subjects.

Because pasteurized milk is low in ascorbic acid and is only a moderately good source of thiamine, a solution containing 65 mg. ascorbic acid and 1 mg. thiamine chloride was added daily to the diet of each individual as well as 1 drop of 10% solution of KI. There were 2 control and 2 test periods for each subject. These were alternated and lasted for about 4 weeks. During the control periods the subjects ate their normal customary diet. No attempt has been made to record the diet during the control periods. There was a 3-day transition before the first test period but for the second test period this was abolished.

All the participants started the milk and honey diet simultaneously. They kept a complete record of the amount of the mixture consumed. The number and the character of stools was also recorded daily as well as the general subjective feeling of each subject.

At the end of each control and test period a thorough physical and dental examination was made, 50 cc. of blood and 24-hour urine samples taken for further study and a capillary fragility test performed. Blood and urine samples were also taken at the middle of each test period. The blood samples were taken before breakfast. The urine samples were kept in a refrigerator during collection and until analyzed.

About the second day of the test period the tongues of all the subjects became coated with a white film and that phenomenon lasted through-

out each test period. As a rule, the bowel movements were normal in all the participants. The feces were light yellow in color and of soft consistency. One subject (W. N.) had occasional difficulty in defecation due to the hardness of the stools. One (E. V.) had diarrhea, of a day's duration 4 times during the first period. Twitching of the eyelid was experienced by some individuals toward the end of the test diet, but this was also observed during the control period. One (W. N.) felt cramps in the stomach several times during the first week of the first test period. Four days before the end of the first test period one of the individuals (R. R.) became ill (common cold). At the middle of the second control period another (E. V.) contracted virus pneumonia. Neither of these subjects participated in the second test period.

**Food Consumption.** On the basis of data presented in the literature Table 1 was constructed, showing composition of the milk and honey for use in computing the consumption of the various food components in which we were interested. The data for the vitamin contents of the honey were taken from the corrected paper by Haydak and co-workers,<sup>18</sup> the original values for the nicotinic acid being divided by 100.

According to the Committee on Food and Nutrition of the National Research Council, the following minimum daily requirements are suggested for adult humans: protein, 70 gm.; Calories, 3000; Ca, 800 mg.; Fe, 12 mg.; vitamin A, 5000 I. U.; thiamine, 1.8 mg.; riboflavin, 2.7 mg.; ascorbic acid, 75 mg.; nicotinic acid, 18 mg.

Table 2 gives the average daily consumption for each individual as well as the consumption of various essential dietary components. It shows that except for nicotinic acid and iron the subjects in the first experimental period ingested more than the minimum daily requirement of all the dietary units for which acceptable standards have been proposed. The iron intake, especially that of AB, was considerably below the normal requirement in both periods. A submaintenance Caloric intake was one of the causes of this inadequacy and was accompanied by a loss of weight.

**Results of Blood and Urine Analysis.** Determinations were made of the calcium, phosphorus, magnesium, ascorbic acid and pantothenic acid contents of the blood plasma. The thiamine, riboflavin and nicotinic acid contents of whole blood were also determined.

For the urine determinations, aliquots from the 24-hour samples were taken. The methods used were: for calcium, Clark and Collip<sup>9</sup> modification of Kramer and Tisdall procedure; for phosphorus, that of Fiske and Subbarow;<sup>13</sup> for magnesium, that of Briggs;<sup>7</sup> for thiamine, that of Hennessy and Cerecedo;<sup>19</sup> for riboflavin, that of Snell and Strong;<sup>42</sup> for ascorbic acid, Bessey;<sup>5</sup> for pantothenic acid, Strong, Feeney and Earle;<sup>46</sup> for nicotinic acid, Snell and Wright.<sup>43</sup>

Table 3 shows that the calcium, magnesium and inorganic P contents of blood plasma were within the limits of normal variation, although the inorganic P tended to be on the lower side of the normal range. However, there is no evidence that this was related to the special diet employed. Robertson<sup>36</sup> gives the variations in Ca and inorganic P content of plasma for 60 normal adults between 20 to 40 years of age

TABLE 2.—AVERAGE FOOD CONSUMPTION PER DAY

Name	Sex	Age	Weight, initial and final (lbs.)	Mixture (cc.)	Milk (cc.)	Honey (gm.)	Protein (gm.)	Calories	Ca (gm.)	P (gm.)	Mg (gm.)	Fe (mg.)	Cu (mg.)	Mn (mg.)	Thiamine* (mg.)	Riboflavin (mg.)	Ascorbic acid (mg.)	Nicotinic acid (mg.)	Pantothenic acid (mg.)	Thiamine per 1000 Cal. (µg.)
<i>First Period: March 1, 1942–March 29, 1942</i>																				
A. B.	F	22	143–138	1900	1769	187	62	1819	2.1	1.6	.2	4.7	.3	.1	1.9	3.5	104.9†	4.4	7.3	1045
M. H.	M	44	145–140	3230	3008	317	105	3090	3.6	2.8	.4	8.0	.6	.2	2.5	5.9	132.7†	7.5	12.4	809
W. N.	M	38	142–150	4275	3982	419	139	4089	4.8	3.7	.5	10.5	.7	.3	3.0	7.8	154.7†	9.6	16.4	734
R. R.	M	25	135–140	4370	4070	428	142	4178	4.9	3.8	.5	10.8	.7	.3	3.1	8.0	156.7†	10.0	16.7	742
E. V.	M	26	134–123	2755	2565	270	90	2534	3.1	2.4	.3	6.8	.5	.2	2.3	5.0	122.8†	6.4	10.5	908
<i>Second Period: May 4, 1942–May 27, 1942</i>																				
A. B.	—	—	139–139	1900	1769	187	62	1819	2.1	1.6	.2	4.7	.3	.1	1.9	3.5	169.9†	4.4	7.3	1045
M. H.	—	—	145–145	3610	3361	354	118	3453	4.0	3.1	.4	8.9	.6	.2	2.7	6.6	234.9‡	8.3	13.8	782
W. N.	—	—	153–158	3990	3716	391	130	3815	4.5	3.5	.4	9.9	.7	.3	2.9	7.3	213.7†	9.1	15.3	760

\* A supplement of 1 mg. of thiamine chloride is included.

† Of this number 65 mg. were added as synthetic ascorbic acid.

‡ Of this number 130 mg. were added as synthetic ascorbic acid (from May 4 to May 19).

§ Of this number 195 mg. were added as synthetic ascorbic acid (from May 20 to May 27).

TABLE 3.—BLOOD AND URINE ANALYSES

Name	Age	Blood analysis (μg., mg. and gm. per 100 cc.)										24 hours urinary excretion										Misc.	
		Hb (%)	Hema-tocrit	Total protein plasma (gm.)	Ca plasma (mg.)	P (inorg.) plasma (mg.)	Mg plasma (mg.)	Thia-mine whole blood (μg.)			Ribo-flavin whole blood (μg.)	Ascorbic acid plasma (mg.)	Pant. acid plasma (μg.)	Nicot. acid whole blood (mg.)	Vol-ume (cc.)	Spec. gravity	Thia-mine (μg.)	Ribo-flavin (μg.)	Ascorbic acid (mg.)	Pant. acid (mg.)	Nicot. acid (mg.)		Pete-chie count (No.)
<i>End of Normal Diet Feb. 18, 1942</i>																							
A. B.	F 22	99	..	..	9.6	3.3	..	6.5	..	..	2	..	..	1000	1010	41.3	..	31.6	..	..	..	..	..
M. H.	M 44	86	..	..	9.1	3.2	..	5.1	..	..	..	..	1650	1020	49.2	..	20.1	..	..	..	..	..	
W. N.	M 38	92	..	..	10.0	3.8	..	7.2	..	..	..	..	1130	1025	50.1	..	40.6	..	..	..	..	..	
R. R.	M 25	78	..	..	10.9	2.9	..	9.2	..	..	..	..	1825	1015	46.4	..	36.7	..	..	..	..	..	
E. V.	M 26	93	..	..	10.2	4.0	..	5.3	..	..	..	..	1000	1025	51.2	..	19.4	..	..	..	..	..	
<i>Honey Diet. Middle of the First Period, March 16, 1942</i>																							
A. B.	..	96	..	..	9.9	3.2	..	6.7	..	..	2	..	..	860	1005	40.2	..	30.2	..	..	..	..	..
M. H.	..	95	..	..	9.6	3.2	..	5.3	..	..	..	..	2070	1010	46.1	..	15.6	..	..	..	..	..	
W. N.	..	92	..	..	10.3	3.8	..	7.8	..	..	..	..	2700	1005	56.3	..	35.2	..	..	..	..	..	
R. R.	..	..	..	..	10.9	2.6	..	10.4	..	..	..	..	2820	1010	40.1	..	30.1	..	..	..	..	..	
E. V.	..	95	..	..	10.6	4.0	..	5.1	..	..	..	..	480	1025	59.6	..	20.9	..	..	..	..	..	
<i>Honey Diet. End of First Period, March 30, 1942</i>																							
A. B.	..	98	47.5	7.9	9.9	3.2	1.5	6.8	50	1.4	16.3	..	..	840	1010	40.6	521.7	30.1	3.4	2.7	57		
M. H.	..	98.5	48.8	7.0	10.0	3.0	2.2	6.4	80	..	6.3	..	..	1200	1015	40.3	401.1	15.4	2.4	3.4	0		
W. N.	..	106	50.5	7.0	10.8	3.8	2.1	7.8	50	1.5	10.4	..	..	2880	1005	57.9	623.2	35.0	3.9	5.5	33		
R. R.	..	87	..	..	11.2	3.3	1.6	9.4	40	..	8.7	..	..	480	1015	53.1	470.3	30.0	1.9	3.2	..		
E. V.	..	106	50.8	8.4	10.9	3.7	1.8	5.4	40	1.0	7.4	..	..	1100	1015	60.3	423.1	18.6	1.9	5.1	4		
<i>End of Normal Diet, April 30, 1942</i>																							
A. B.	..	90	42.0	7.8	9.3	3.3	1.6	6.4	40	..	3	16.0	..	820	1005	..	990	35.3	3.8	..	23		
M. H.	..	91	45.1	7.7	9.0	3.4	2.1	6.2	60	1.0	6.1	..	..	880	1025	..	1554	19.3	2.2	..	0		
W. N.	..	96.5	47.2	7.9	10.2	3.8	2.1	7.6	40	..	3	10.9	..	1175	1015	..	470	41.6	4.0	..	7		
<i>Honey Diet. Middle of the Second Period, May 20, 1942</i>																							
A. B.	..	88	..	..	9.9	3.0	1.7	6.8	50	..	3	14.3	..	880	1005	40.1	517	31.7	3.4	2.3	14*		
M. H.	..	91	..	..	9.8	3.0	2.2	6.9	60	..	4	6.0	..	2420	1010	47.3	462	17.6	2.1	2.7	1		
W. N.	..	97	..	..	10.9	3.4	2.0	8.3	40	..	5	10.6	..	2410	1015	56.4	633	40.0	3.7	5.8	2*		
<i>Honey Diet. End of the Second Period, May 27, 1942</i>																							
A. B.	..	83	41.8	7.0	10.1	3.0	1.7	6.2	60	..	3	14.4	..	500	1005	39.3	530.7	32.6	3.6	3.0	2†		
M. H.	..	95	47.3	7.1	9.8	3.0	2.3	7.3	70	..	4	6.4	..	2700	1005	51.2	470.1	16.2	2.2	2.8	0		
W. N.	..	97	48.0	6.8	10.9	3.6	2.0	8.3	50	..	6	10.1	..	2280	1010	54.6	620.6	39.3	4.0	5.1	0		

\* 130 mg. synthetic ascorbic acid was added to the diet.  
† 195 mg. synthetic ascorbic acid was added to the diet.

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as 9.9 to 11.1 mg./100 cc. and 3.1 to 4.8 mg./100 cc., respectively. Hirschfelder<sup>20</sup> considered the normal variation for the plasma Mg. to be from 1.8 to 2.5 mg./100 cc.

From the table it is also evident that the blood levels for thiamine were within the limits of normal variation. Guhr,<sup>15</sup> Ritsert,<sup>35</sup> Rowlands and Wilkinson,<sup>38</sup> Sinclair<sup>41</sup> found these limits to be between 3 and 16.5 micrograms per 100 cc. and as Guhr has pointed out, the physiologic variations are larger, since some apparently normal individuals have no more than a trace.

The daily urinary excretion of thiamine in our investigation was considerably lower than the normal levels given in the literature, in spite of the fact that the daily intake was adequate according to present standards. A number of investigators<sup>8,27,28</sup> found the normal 24-hour urinary excretion to vary from 60 micrograms to more than 100 micrograms. Patients suspected of thiamine deficiency excreted 5 to 70 micrograms daily.

The blood levels of riboflavin were within the limits of normal variation which were found to be from 0.35 to 0.5 micrograms/cc.<sup>2,47</sup> Daily urinary excretions of riboflavin were also within the reported limits of normal variation (500 to 800 micrograms).<sup>40,47</sup>

The blood plasma level of ascorbic acid in our subjects was generally low but nevertheless was mostly in the reported lower limits of normal variation. Several investigators<sup>6,12,14,45,48</sup> found the limits to be from 0.3 to 1.74 mg./100 cc. However, as pointed out by Storvic and Hauk,<sup>45</sup> "A mean plasma value which is indication of saturation for one subject may signify partial depletion of stores for another."

Although the average daily excretion of ascorbic acid in our subjects was rather low, it still was within the limits of normal variation reported. Levovich and Batchelder<sup>24</sup> found it to be from 6 to 29 mg., with the intake ranging from 27 to 253 mg. Storvic and Hauk<sup>45</sup> reported an increase in the daily urinary ascorbic acid excretion from 26 mg. on a 50-mg. intake to 130 to 164 mg. on a 200-mg. daily intake.

In our experiment there was no correlation between the ascorbic acid intake and the blood plasma level or the urinary excretion of ascorbic acid. At the beginning of the second test period A. B. and W. N., because of the high petechiæ count, received a daily supplement of 130 mg. of ascorbic acid. The blood and urine analysis at the middle of the period did not show any response to this change. Because the petechiæ count of A. B. was still high, 195 mg. of ascorbic acid were added daily to her diet. This addition did not manifest itself in any way in the subsequent blood and urine analysis.

There was no correlation between the number of petechiæ and either the level of ascorbic acid in the blood plasma or the 24-hour urinary excretion. However, the number of petechiæ diminished with the increased dose of ascorbic acid. It is of interest to note that Levovich and Batchelder<sup>24</sup> found that, "The capillary resistance was not significantly affected by varying the amount of crystalline ascorbic acid added to the vitamin C free diet."

The pantothenic acid content of plasma in our experiment was,

except in the case of A. B., considerably lower than some values given in the literature. Stanbery *et al.*<sup>44</sup> found that the pantothenic acid content of whole blood of 18 normal persons varied within fairly narrow limits, 0.19 to 0.32 micrograms per cc. The blood of 28 patients with pellagra, beri-beri, and riboflavin deficiency showed a decreased content averaging 23% to 50% below the normal. According to Pearson,<sup>32</sup> the blood plasma pantothenic acid content of humans is  $17 \pm 3.3$  micrograms per 100 cc. However, Pelczar and Porter,<sup>33</sup> employing a different microbiologic technique found the pantothenic acid content of whole blood of 17 normal persons to range between 0.03 and 0.099 micrograms per cc. (av., 0.059). In view of the recent suggestion by Wright<sup>50</sup> that the low value obtained by Pelczar and Porter<sup>33</sup> are due to failure to determine the "combined" fraction, constituting the major portion of the total pantothenic acid in the blood, which fraction can be released by autoclaving, it may be stated that the samples of blood analyzed in the present study were subjected to autoclave temperature. Thus the values reported cannot be legitimately compared with the Pelczar-Porter normals.

The 24-hour urinary excretion of pantothenic acid was quite constant for each individual and apparently was not influenced by the amount of the vitamins ingested. The excretion found is within the range of 1.46 mg. to 6.79 mg. reported by Pelczar and Porter.<sup>33</sup>

The nicotinic acid content of the blood was, except in the case of W. N., lower than the normal levels reported for this vitamin. Melnick and co-workers<sup>29</sup> give the nicotinic acid values in the whole blood of normal adults as 0.54 mg. to 0.84 mg. per 100 cc. for males and 0.52 to 0.74 mg. per 100 cc. for females. The average values given by Kochhar<sup>22</sup> and Bandier<sup>4</sup> are somewhat lower, namely  $0.37 \pm 0.13$  mg. per 100 cc. and 0.37 mg. per 100 cc. respectively. The nicotinic acid intake in our experiment was considerably below the suggested minimum requirement for that vitamin.

The daily urinary excretions of nicotinic acid were near the lower limits of the normal variations reported. The ranges found by various workers for normal individuals are as follows: Bandier,<sup>3</sup> 1.5 to 5 mg.; Raymond,<sup>34</sup> 3 to 5 mg.; Rosenbloom and Jolliffe,<sup>37</sup> 3.4 to 10.2 mg.; Melnick *et al.*,<sup>30</sup> 1.7 to 29.3 mg.

**Dental Study.** A thorough examination of the subjects' mouths was made on February 19, 1942. Cavities, relation of the bite, mobility of teeth and especially the color, form and texture of the gums were recorded. Also bite-wing Roentgen rays of the posterior teeth and one regular dental film of the lower anterior teeth were taken.

On March 27 the mouths were again examined and another set of Roentgen rays taken. A study of the radiographs showed no radiographic changes. Of the 5 mouths examined, 1 (A. B.) showed no clinical changes from the first examination on February 19. The other 4 mouths had a heavy, white, cheesy film covering the teeth and gums. Two of these subjects had a heavy coating of this film on the inside of the cheeks and on the tongue. All 4 had a general hyperemia and redness of the marginal gingiva throughout the whole mouth. The

areas recorded in the first examination (February 19) as hyperemic and red were more aggravated and were a brighter red in color. Also these areas had a more shiny surface and bled easily when probed. No change in gingival form was evident. The tongue of one of the subjects was marked with small red patches circumscribed with a white raised border.

On May 28 (the end of the second test period) the mouths were again examined and another set of Roentgen rays taken. Again no radiographic changes were revealed. Of the 3 mouths examined the one showing no changes at the end of the first period again showed no clinical symptoms. The subject having the very heavy film on cheeks and tongue was advised to brush the teeth twice daily before beginning the second month of the experiment. This reduced greatly the white cheesy film present on the teeth and the soft tissues. There was less redness and hyperemia of the gingival tissues than at the end of the first test period. The third subject again showed red patches on the tongue; also a heavy accumulation of the white cheesy material around the necks of the teeth, with edema and marked redness of the lower anterior gum. The gums bled profusely when only wiped and cleaned with cotton.

Of interest was the fact that brushing did not seem to remove the heavy white film from the teeth after it once formed. However, as soon as the subjects returned to a normal diet this film disappeared. The same held true of the tongue having red patches; the patches disappeared on the second day after the normal diet was resumed. Also noteworthy was the fact that 2 of the men experienced a feeling of numbness and soreness of the teeth when first biting and masticating solid food.

The development of the hyperemic, red condition of the gingival margins of the teeth is probably explained: (a) by chemical and bacteriologic irritation from the film that covered the teeth and gums; and (b) by lack of stimulation and mechanical cleansing of the gums normally derived from the mastication of solid food.

**Summary.** Five healthy individuals varying in age from 22 to 44 years were put on a daily diet consisting of a mixture of 1 quart of pasteurized milk with 100 gm. of light honey. A solution containing 65 mg. ascorbic acid and 1 mg. thiamine chloride was added daily to the diet of each individual. There were 2 control and test periods lasting about 1 month each.

The hemoglobin, Ca, inorganic P, Mg, thiamine, riboflavin, ascorbic acid, pantothenic acid, and nicotinic acid contents of the blood and the 24-hour urinary excretion of the vitamins were determined at the end of each period.

The results showed that the amounts of most of these constituents in the blood were within the limits of the normal variations given in the literature. The ascorbic acid values of the blood plasma were, in general, low, but mostly within the reported limits of variation for normal adults. The pantothenic acid content of blood was low for most of the subjects as judged by the more acceptable work of other



investigators. Most of the nicotinic acid values obtained were subnormal which is in line with the sub-standard intake of this vitamin.

There was no correlation between the intake of ascorbic acid and the level of this vitamin in the blood or its excretion in the urine. The 24-hour urinary excretions of ascorbic acid were normal, although rather low, in spite of a rather large intake of ascorbic acid supplement.

No correlation could be found between the number of petechiæ and the amount of ascorbic acid in the blood or its excretion in the urine. However, there was a correlation between the number of petechiæ and the amount of crystalline ascorbic acid added to the diet.

The 24-hour urinary excretions of riboflavin and of pantothenic acid were normal and that of nicotinic acid near the lower limits of the suggested normal range without any special supplements of these vitamins. The excretion of thiamine was definitely subnormal in spite of an adequate intake provided in part by thiamine chloride supplement to the milk-honey mixture.

The hyperemic condition of the gums observed during the test diet was probably not due to dietary deficiency. It could be explained by the physical character of the diet which did not provide for any mastication.

**Conclusion.** This diet proved to be adequate to support life, but not to prevent deficiency symptoms entirely.

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## THE DEVELOPMENT OF PULMONARY TUBERCULOSIS IN CONGENITAL HEART DISEASE

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It is a commonly accepted premise that those individuals suffering from congenital heart disease, notably pulmonary stenosis, are particularly prone to develop, and later, to succumb to pulmonary tuberculosis. This premise is based chiefly on statistics compiled from case reports in the literature, 2 of the most complete tabulations being those of Norris<sup>15</sup> (1904) and Abbott<sup>2</sup> (1936). There are, however, few reports of specific cases in which congenital heart disease and pulmonary tuberculosis existed concomitantly, and fewer still which explain the evolution of the pulmonary infection in relation to the cardiac anomaly. It is the purpose of this study to investigate the frequency of congenital heart affections in a tuberculosis institution, to determine the type and course of the pulmonary disease and to evaluate the efficacy and advisability of collapse therapy in the face of the cardiac handicap.

**Material.** The material for the report includes 13 cases of congenital heart disease, upon 7 of which postmortem examinations were performed. These 7 cases were discovered during the course of 1545

examinations on tuberculous individuals, an incidence of 0.4%. This incidence may be higher than that encountered in other tuberculosis institutions since 250 of our 2000 beds are allotted to the pediatric service. It is considerably lower than that reported from institutions or services limited entirely to the treatment of children (5.5% Gelfman and Levine<sup>10</sup>). It is approximately the same in general hospitals, if deaths under 2 years of age due to congenital cardiac anomalies are excluded (0.5% Gelfman and Levine;<sup>10</sup> 0.4% Cabot;<sup>5</sup> 0.9% Rannels and Propst<sup>16</sup>).

Ten of our patients were males, 3 females, giving a ratio essentially the same as in our general autopsy material. It is interesting that 6 patients were of Italian descent. Since, however, we had no information concerning consanguineous marriages or other hereditary factors, it is impossible to suggest an explanation. There is no predominance of Italians in either our hospital census or our autopsy material.

**Frequency.** It is now almost 100 years since Rokitsansky<sup>18</sup> observed that pulmonary tuberculosis and heart disease are incompatible, particularly when the latter is associated with cyanosis. He began a controversy which still continues. Most observers now insist that, since the cyanosis of pulmonary stenosis differs in its origin from that of acquired valvular lesions, the two diseases are not mutually exclusive and, in fact, that pulmonary stenosis actually favors the development of tuberculosis. In Abbott's<sup>2</sup> analysis of 1000 cases of congenital heart disease, 110 revealed pulmonary stenosis. Of these, 24 (22%) are listed as having tuberculosis. Of the remaining 890 cases with various types of congenital defect, 50 (5.5%) had tuberculosis. The next highest incidence of tuberculosis after pulmonary stenosis occurred in the group of 62 cases with interventricular septal defects (14.5%). Other authors report an incidence of pulmonary tuberculosis in pulmonary stenosis varying from 10% to 80%.<sup>3,11,19,21</sup> To assist in clarifying this question of incidence, we selected at random from the literature of the past 10 years, 50 cases of congenital heart disease, in which death occurred after 5 years of age and in which necropsy records were complete. In this group, 2 of 21 cases (9.5%) with pulmonary stenosis and 1 of 18 cases (5.5%) with interventricular septal defect succumbed to tuberculosis.

Our own cases support the view that patients with pulmonary stenosis are especially susceptible to pulmonary tuberculosis. All 7 of those which came to necropsy demonstrated this defect, in 5 of which it was associated with the other components of the tetralogy of Fallot. Of the 6 cases which were not autopsied, 3 were clinically diagnosed pulmonary stenosis. One of these was proved by contrast visualization of the cardiac chambers by the multiple fluorographic and motion picture technique and has been reported by Grishman, Steinberg and Sussman.<sup>13</sup> Of the remaining 3 cases, 1 had a patent ductus arteriosus and the others were diagnosed non-cyanotic congenital heart disease, without evidence of pulmonary stenosis.

Apparently, then, of all congenital cardiac anomalies those patients with pulmonary stenosis are most likely to develop pulmonary tuber-

culosis. Whether they are more likely to develop phthisis than are individuals with normal hearts is impossible to determine without additional statistical data. Since, the mortality rate from tuberculosis in the general population differs with age, sex, race, locality, and other factors, information regarding each of which is not available in the reported cases of congenital heart disease, there can be no basis for comparison of the two groups. The fact that our cases fall into a younger age group than is usual in our autopsy material indicates that there may be a measure of predisposition in patients with pulmonary stenosis. The youngest patient was 9, the oldest 60. Seven cases were from 4 to 20 years of age, a preponderance which is about a decade younger than the highest mortality usually reported from tuberculosis.<sup>9</sup>

Whether patients with this congenital anomaly are more likely to die of tuberculosis than any other catastrophe is a little easier to determine. In Stölker's<sup>19</sup> series, of those who lived beyond 19 years just as many succumbed to a cardiac death as to tuberculosis. In Abbott's group of 110, there were almost twice as many cardiac deaths as there were patients listed as having tuberculosis. She has noted<sup>1</sup> that the incidence of tuberculosis rises as the age group increases, an observation, however, which can be noted in any group of individuals. In the cases mentioned above which we have culled from the literature, death was due in many more instances to other causes, than to phthisis.

It is obvious, then, that while tuberculosis is a hazard to the patient with pulmonary stenosis, it is a no greater menace than his own cardiac defect. This fact also explains why the incidence of pulmonary stenosis in a tuberculosis institution is no higher, if as high, as in a general hospital. Many of these patients apparently succumb before they have an opportunity to develop tuberculosis or are so incapacitated by their cardiac defect that they are protected from infectious contacts.

**Clinical Observations of Cardiac Disease.** The symptoms and physical examination of the cardiovascular system in our patients were typical of the congenital defect found at autopsy examination, the specific defect being diagnosed correctly before death in every instance in which exact classification was attempted. With the help of Roentgenography and fluoroscopy the diagnoses were unusually accurate. Since most of these defects exemplified the tetralogy of Fallot a composite picture covers most points of interest.

**Case Study.** The patient is a white youngster in his lower 'teens. The history reveals cyanosis from birth or shortly thereafter with the diagnosis of congenital heart disease made in infancy or early childhood. The youngster has been placed on restricted or moderate activity, depending upon his subjective symptoms. He has been followed in a hospital out-patient department and has had at least one previous hospital admission for observation of the cardiac defect. There has been no evidence of congestive failure and he has led a fairly normal life until the onset of his pulmonary disease. Examination reveals an underdeveloped child who appears younger than his chronologic age. He is cyanotic and there is moderate to marked clubbing of the fingers and toes. Dyspnea is not marked. The heart is enlarged in all dimensions and there is a loud, harsh systolic murmur heard best at the base, usually associated with a systolic thrill. Abnormal findings in the lungs are dependent upon the pul-

monary pathology. Routine laboratory studies reveal a well-marked polyeuthemia and sputum is positive for tubercle bacilli. The electrocardiogram reveals right axis deviation with tall P waves, which are often notched. Roentgenography and fluoroscopy demonstrates enlargement of both left and right ventricles with frequently a sharply convex right border indicating marked enlargement of the right ventricle. The pulmonary conus is usually prominent. This is contrary to Grier's<sup>12</sup> observations, probably because his studies were made in a younger age group than ours. The venous pressure is within normal limits. The blood pressure tends to be normal or slightly decreased.

Up to this point, then, it is obvious that the pulmonary disease has not influenced the classical findings in cases of tetralogy of Fallot. The two cases exhibiting other cardiac anomalies, *cor biatricatum triloculare* and pulmonary stenosis with patent foramen ovale, likewise do not deviate from the typical pattern. It is interesting that the triloculate heart, as well as the case of tetralogy of Fallot that was proved by contrast visualization of the cardiac chambers, both demonstrated equal saccharine and ether circulation times. This has been suggested as a convenient method for determining the presence of an arteriovenous shunt (Grishman<sup>13</sup>). Unfortunately in only a few of our patients was this test performed. In one tetralogy of Fallot, the ether and saccharine times were 9 and 15 seconds respectively. In the case of patent ductus arteriosus they were 17 and 25 seconds respectively.

The vital capacity was not obtained in many of our patients, but where this determination was made, it was usually diminished but within normal limits. In the case of tetralogy of Fallot reported by Grishman *et al.*,<sup>13</sup> these authors found the vital capacity to be 3500 cc. This was before the onset of the pulmonary tuberculosis. A few months after onset, it had fallen only 300 cc. The lowest vital capacity in our series was in a patient with tetralogy of Fallot in which it was 950 cc. with a Peabody factor of 3, interpreted as 30% of the normal resting minute ventilation. This determination, however, was made following reexpansion of a successful therapeutic pneumothorax, in which the collapsed lung was fibrotic and in which the contralateral lung was the site of a fresh spread. Vital capacities in other patients with the tetralogy of Fallot were 2500 (Peabody factor, 6), 2700 (Peabody factor, 7.9) and 2500 (Peabody factor, 5.2).

**Cause of Death.** Congestive failure was a prominent feature only in the case of patent ductus arteriosus. It was present in 3 other cases terminally or during the last few months of life. Bacterial endocarditis was found in 3 of the cases which were examined at postmortem, but was neither extensive nor even a contributory cause of death. Two of these cases had fibrinous pericarditis, 1 of minimal and the other of moderate degree. With the exception of 1 case which succumbed from extension of a previously resected brain tumor, the immediate cause of death in all our patients was progressive pulmonary tuberculosis. In 3 cases who died but did not come to autopsy, the progressive pulmonary involvement was likewise the apparent determining factor terminally.

**The Pulmonary Aspect.** In 4 of the 13 cases the presence of close contact of the patient with an open case of pulmonary tuberculosis

was established. In 2 other cases there had been frequent hospitalizations on general wards, which may have been an exposure factor. In any event, at least 4 of our patients had a good chance to develop tuberculosis irrespective of their cardiac condition. In the literature there is ordinarily no notation made of contacts, whether absent or present. When such observation is made, it is usually in the negative.<sup>4,8</sup>

The onset of pulmonary disease in our cases was similar to that of patients without congenital heart disease. In 3 cases the tuberculosis was ushered in with hemoptyses, although in none of these had hemoptyses previously been a part of the cardiac picture. In 1 case hemoptyses had been present for several years before and up to the onset of tuberculosis and it was impossible to determine the etiology of the later bleeding, whether cardiac or pulmonary. In another case, hemorrhage had been a prominent feature in the boy's earlier life, but had ceased several years before the onset of the pulmonary infection. Whether or not an hemoptysis was the presenting symptom of the tuberculosis, some or all of the usual symptoms of this disease were always present. Weight loss, night sweats, easy fatigability, fever, productive cough, and chest pain were present in varying degrees in each case. The severity of these symptoms was necessarily influenced by the degree to which they had been present due to the cardiac disease. The fatigability was greater than the patients had usually experienced. Chest cardiac pain when present previously became lancinating in character and was influenced to a greater extent by respiration. If a chronic cough were present, it became productive of purulent sputum. In no case did the patients notice at the onset of the pulmonary infection an increase in their dyspnea and cyanosis.

The difficulty of differentiating between congenital heart disease and pulmonary tuberculosis and in diagnosing the latter in the presence of a cardiac lesion has been stressed in some of the studies of the two diseases.<sup>7</sup> Such difficulty was not encountered in our cases, since the congenital anomaly was diagnosed in all cases many years prior to the onset of the pulmonary infection, which was recognized fairly promptly at its onset. This is probably due to the fact that many of these patients were followed closely from the cardiac aspect with frequent fluoroscopic examinations. In spite of this fact, the pulmonary disease on admission, in all but 1 case, was a moderately or far-advanced one, as is true of most of our admissions.

The course of the disease was also similar to that observed in most of our patients who are admitted without the added cardiac hazard. The duration of life apparently depended, as in all tuberculous patients, upon the extent of the disease on admission and the effectiveness of collapse therapy, when that form of treatment was utilized. A glance at Table 1 will reveal how little the length of life depended upon either the age of the patient, the congenital abnormality, or the presence of cyanosis. The longest duration (6 years) was in a boy of 15 with the tetralogy of Fallot who had marked cyanosis from birth. The shortest courses (7 months) were in a man of 27 with the tetralogy

of Fallot and cyanosis since birth and a boy of 21 with a *cor biatriatum triloculare* and cyanosis from the age of 4 years. The average duration of life from the onset of the pulmonary infection to exitus was 1 to 2 years.

TABLE 1.—ANALYSIS OF CASES

Case	Sex	Age	Abnormality	Cyanosis	Duration of Tb.	Pneumothorax	Congestive failure	Outcome
1	M	9	Tetralogy of Fallot	Marked from 4 yrs.	2 yrs.	0	0	Autopsy
2	M	14	Tetralogy of Fallot	Marked from 1 yr.	9 mos.	0	0	Autopsy
3	M	21	Cor biatriatum triloc.	Marked from 4 yrs.	7 mos.	0	Present	Autopsy
4	M	11	Tetralogy of Fallot	Marked from birth	15 mos.	0	0	Autopsy
5	M	15	Tetralogy of Fallot	Marked from birth	6 yrs.	Effective	0	Autopsy
6	F	11	Tetralogy of Fallot	Moderate	16 mos.	Ineffective abandoned	0	Autopsy
7	F	21	Pul. stenosis and patent for. ovale	Terminal	4 yrs.	Ineffective; abandoned	Terminal	Autopsy
8	M	33	Pat. ductus arteriosus	On exertion	1 yr.	0	Present	Discharged; arrested
9	M	60	Congenital heart dis.	Minimal	3 yrs.	0	Present	Signed out; improved
10	M	19	Prob. pul. stenosis	On exertion	5 yrs.	Ineffective; abandoned	0	Signed out; unimproved
11	M	27	Tetralogy* of Fallot	Marked from birth	7 mos.	0	0	Died; progressive pulm. Tb.
12	M	19	Prob. tetralogy of Fallot	Marked from birth	2½ yrs.	Ineffective; abandoned	0	Died; progressive pulm. Tb.
13	F	16	Congenital heart dis.	On exertion	8 mos.	0	0	Died; progressive pulm. Tb.

\* Proved by contrast visualization of cardiac chambers (Grishman *et al.*<sup>13</sup>).

The pulmonary tuberculous lesions, as well as the type of onset and course of the disease, were similar to those usually observed in our autopsy material. In every case which came to necropsy, there was a typical adult type, chronic pulmonary tuberculosis of the lungs with numerous excavations, frequently interlocking, areas of caseous lobular pneumonia, and bronchogenic acinous nodose spread to most or all lobes. Gastro-intestinal involvement with tuberculous ulcerations, which we have usually found in this type of pulmonary disease, was present in 6 of the 7 cases. Laryngeal tuberculosis, which we have also found a frequent though later associate of chronic pulmonary tuberculosis, was present in 2 of the 7 cases.

As the lesions and other factors of the pulmonary infection are the same, whether or not the patient has congenital heart disease, and as the cause of death depends rather on the pulmonary than on the cardiac status, it is the lung rather than the heart which should be the focal point of therapy. Bed rest may be sufficient treatment for the lung and useful to the heart, but when it fails to arrest the progression of the tuberculous infection or cannot accomplish cavity closure, it must in spite of the heart be supplemented by collapse therapy. Frequently such therapy has been postponed, not because the pulmonary involvement was unsuitable for collapse, but because the clinician was reluctant to place an added burden on the defective cardiac system. When instituted late in the course of the disease, collapse therapy is neces-

sarily a "do or die" procedure and the possibility of arresting the course of the tuberculosis is doubtful indeed. Admittedly, the life expectancy in these patients, even without the added pulmonary com-

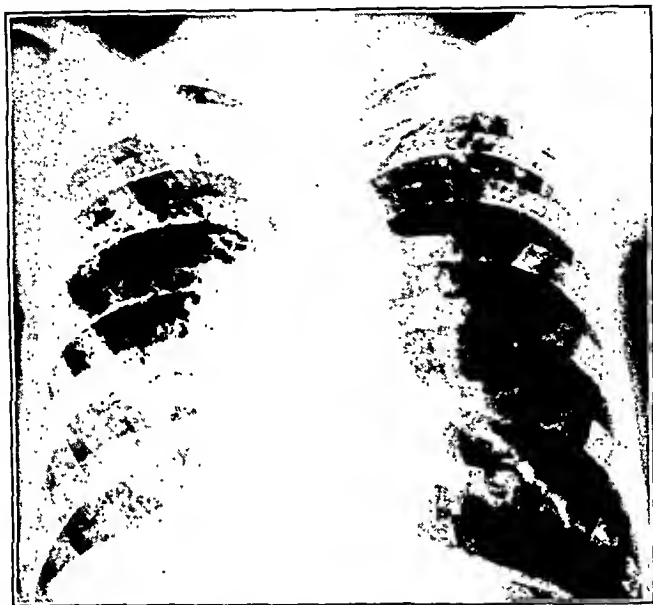


FIG. 1.—Case 3. Chest roentgenogram 1 month before death. Bilateral upper lobe excavations with extensive bronchogenic spread throughout the right lung. Prominence of the aortic knob. Straight right cardiac border.

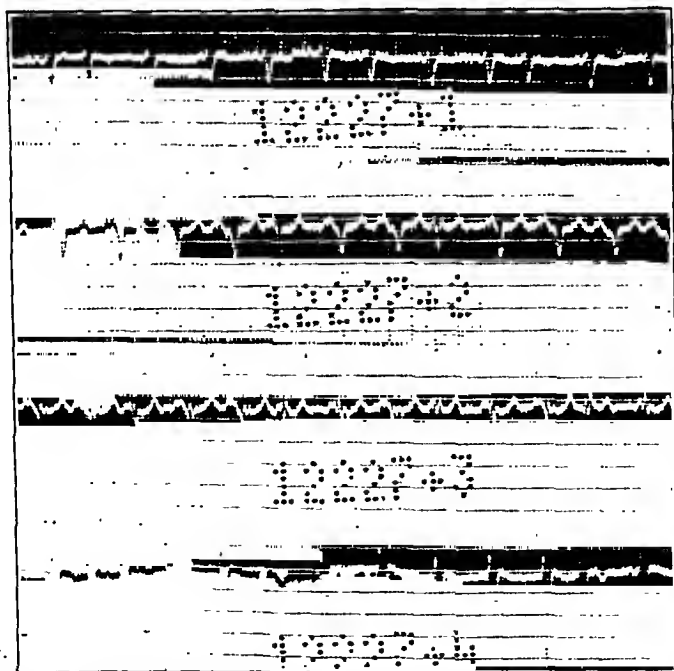


FIG. 2.—Case 3. Electrocardiogram 2 weeks before death. Right axis deviation. P waves notched. Many premature contractions.



plication, is short. Nevertheless, measures which may be taken to prevent the patient from succumbing to tuberculosis, although they may be hazardous ones, seem justified. Because these patients are tuberculous is no reason why they cannot be given the opportunity to emulate Paul White's<sup>22</sup> famous musician who lived with a tetralogy of Fallot into his 60th year.



FIG. 3.—Case 3. Complete absence of the interventricular septum. Probe A passes from the ventricle into the pulmonary artery. Probe B passes through the widely patent foramen ovale. Probe C passes from the ventricle into the aorta.

This attitude towards active therapy is reflected in the literature of the past 10 years. Ritschel<sup>17</sup> in 1932 reported a case of pulmonary stenosis with marked cyanosis in which he instituted pneumothorax and secured cavity closure and arrest of the tuberculous infection. He noted no increase in the clinical signs of heart disease after the induction of pneumothorax and concluded that congenital pulmonary stenosis was not a contraindication to this form of therapy. Hille-sheim<sup>14</sup> also induced pneumothorax in cases with pulmonary stenosis and secured satisfactory results without cardiac complications. We have discovered no case in the literature in which thoracoplasty was done to control the pulmonary lesion.

In our cases pneumothorax was instituted in 5 cases. In 3 of these the pneumothorax was abandoned as ineffective because of adhesions which could not be severed by closed pneumonolysis. In 1 case the pneumothorax was abandoned because of adhesions and the develop-



FIG. 4.—Case 5. Chest roentgenogram. Lesion on the right controlled by pneumothorax. Excavations in the left upper lobe. Heart is boot shaped. Fluoroscopy revealed the bulge on the right to be pulsating, apparently ascending aorta.



FIG. 5.—Case 5. Chest roentgenogram 5 years later. Right lung has been expanded and postmortem examination revealed a healed excavation. There has been progression of the lesion on the left.

ment of empyema. In this same case pneumothorax was induced on the contralateral side, as a last resort measure, and death occurred too soon after induction to accomplish cavity closure. In 1 case pneumothorax accomplished good results and the lung was reexpanded. Postmortem examination revealed a closed cavity on the pneumothorax side, death resulting from extensive cavernous disease in the contralateral lung. In no case did collapse therapy increase the cardiac symptoms or lead to congestive heart failure.

In reviewing the therapy which our patients received, there are at least 2 cases in which treatment was probably inadequate. Both of these patients were admitted with essentially unilateral pulmonary disease for which pneumothorax was induced, but later abandoned as ineffective. In the first case a thoracoplasty was then advised. The procedure was not acceptable to the patient, he developed a spread in the contralateral lung and expired  $2\frac{1}{2}$  years after the onset of the pulmonary infection. In the second case, a thoracoplasty was considered, but the operation was never done because the cardiac condition was thought to be too great a hazard. It is interesting that in this case, the cardiac consultant did not object to the major surgery, provided no other less drastic methods of treatment were available.

In general, the indications for thoracoplasty in our hospital include a contralateral lung free of disease or containing a controlled lesion and a vital capacity equal to at least 50% of the normal resting minute ventilation. We do not feel that in this group with congenital heart disease that these indications should be altered, unless there is evidence of congestive cardiac failure. The immediate postoperative condition will always depend upon the degree of shock, the paradoxical respiration, diminution of vital capacity and spread of disease from aspiration of infected material. The first two complications can be treated. The third need not necessarily be great, since the portion of lung which is collapsed is to a large extent useless as respiratory tissue due to the disease process. In any case, this selective type of collapse is probably more nearly accomplished with a thoracoplasty than a pneumothorax, since in the latter, large portions of good pulmonary tissue must often be compressed in order effectively to close excavations elsewhere in the lung. The danger of contra- or homolateral spreads from aspiration of infected material is always present and, to a large extent, uncontrollable, whether or not a cardiac lesion is present. In view of these facts, we can recommend that congenital heart disease should not be considered a contraindication to thoracoplasty and in order not to waste the few years of life expectancy of these patients immediate operation may be more advantageous than a preliminary trial of pneumothorax.

The newer mechanisms of cavity closure, such as Monaldi and cavernostomy, may also be of particular value to these patients. In these procedures where the excavation is attacked directly, there is practically no diminution in vital capacity and since the operation is not a formidable one, there is no shock. The dangers of spread are also minimized since there is a direct drainage of infected material from

the cavity. However, these techniques are too new to permit adequate evaluation and certainly not all types of cavernous disease are suitable for these methods of attack.

**The Physiologic Relationship of a Pulmonary and Cardiac Disease.** Since we do not have sufficient knowledge to determine all the factors necessary for the development of tuberculosis in individuals with normal cardiovascular systems, speculation concerning how and why it develops in patients with congenital heart disease must necessarily be nebulous. Factors leading to the greater susceptibility to tuberculosis of patients with pulmonary stenosis have been variously stated to be due to anemia of the pulmonary vascular bed; lowering of resistance due to the accumulation of the toxic products of metabolism that escape oxygenation; the life expectancy which extends to the age group most susceptible to tuberculosis;<sup>1</sup> and the unequal pulmonary and bronchial circulations.<sup>15</sup>

As, however, there are certain definite metabolic characteristics in the hemorespiratory exchange in morbus cœruleus one is forced to the conclusion that, whatever predisposition to tuberculosis exist in these cases, it must be based primarily upon those changes. Talbott, Coombs, Castleman, Chamberlain, Consolazir and White<sup>20</sup> in 1 case, and Campbell, Hunt and Poulton<sup>6</sup> in 3 cases of morbus cœruleus, found consistent metabolic factors. There was an increased O<sub>2</sub> capacity, decreased O<sub>2</sub> saturation, decreased carbon dioxide content, decreased partial pressure of alveolar carbon dioxide, and decreased arterial pH. The acidosis is particularly interesting since it was probably due chiefly to the concentration of acids in the blood, acids which are not definitely identified, but which are organic and in all likelihood the toxic products of metabolism.

Although metabolic studies were not as complete in our cases as in those mentioned above, those that were done agree with the findings of these authors. In 2 cases with the tetralogy of Fallot the arterial oxygen was 26 cc. and 23 cc. per 100 cc. of blood respectively. The arterial carbon dioxide was 34 cc. and 34.6 cc. per 100 cc. of blood. In 1 case of pulmonary stenosis the arterial oxygen was 25 cc. and the carbon dioxide was 42. Since these changes are so typical of this group of cases, their apparent susceptibility may well be due to this variation from the normal. Exactly how this predisposition functions and in what respect the altered tissue physiology furnishes suitable culture media for the tubercle bacillus is impossible to determine.

**Summary and Conclusions.** A study has been made of 13 patients who had congenital heart disease and contracted pulmonary tuberculosis, upon 7 of whom postmortem examinations were performed. The most common congenital defect was pulmonary stenosis, which was present in all cases which came to autopsy, implying a certain predisposition of these patients to tuberculosis.

A composite picture of the typical case, including clinical, physiologic and pathologic studies, has been presented. From this material we may conclude that the pulmonary tuberculosis runs a course typical of that disease, irrespective of the cardiac lesion. The functioning of

the defective cardiac system is affected unusually little by the superimposed respiratory infection.

In view of this observation, together with the fact that our patients succumbed to the pulmonary involvement rather than failure of the defective cardiovascular system, active treatment of the tuberculosis is recommended. Pneumothorax induced in 5 of our patients did not lead to congestive heart failure in any instance. The importance of other surgical forms of collapse therapy for these patients has been discussed.

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## A CASE OF HISTOPLASMOSIS IN AN INFANT WITH AUTOPSY

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At the time of Darling's<sup>1</sup> first description of histoplasmosis (1906), he thought that the infectious agent was a protozoan related to members of the genus *Leishmania*. In 1934 Dodd and Tompkins<sup>2</sup> from Tennessee announced the 6th case on record and supplied DeMonbreun with material from which he cultured the organism.<sup>3</sup> Though appearing as a yeast form in the human host, on artificial media it grew as a

mass of matted mycelial threads with prominent chlamydospores. Thus was disproven the protozoan etiology of this disease which even today startles the pathologist from the humdrum of routine by its resemblance to kala-azar. In 1939 the number of cases recorded had risen to 12 and by 1941 had reached a total of 32. At present over 50 have been reported, ranging widely in geographic distribution, as well as in age and sex incidence.

In a recent report VanPernis, Benson and Holinger<sup>4</sup> summarized the various pathologic lesions found in the disease and added a case of their own in which the presenting symptoms were produced by invasion of the larynx. Prominent in all is involvement of the hemolytotoxic system: the lymph nodes, particularly the cervical, tracheobronchial, mesenteric, and periaortic nodes; the follicles of the lungs and gastro-intestinal tract; the spleen; and the bone marrow. In these structures the mononuclear and endothelial cells are heavily parasitized. The liver is usually enlarged, the parenchymatous as well as the Kupffer cells contain the fungus. Invasion of either or both the cortex and medulla of the adrenal glands is not uncommon. Less frequent is involvement of the heart, kidneys, thymus, pancreas, skin, meninges, pharynx, and joints.

Grossly as well as microscopically the lesions resemble those of tuberculosis; a central area of necrosis surrounded by large monocytes and lymphocytes. The Langhans giant cells are usually absent. The presence of the yeast form of *Histoplasma capsulatum*, usually most numerous in the monocytes, is the outstanding distinguishing characteristic. The present case conforms well with the "typical" pathologic picture as developed during the past decade. The only unusual feature is the extreme youth of the patient.

**Case Report.** A boy, 7 weeks old, was admitted to the hospital and died 2½ weeks later. Delivery had been normal after 8 months gestation, weight at birth being 5 pounds. Though never breast-fed, in growth and appearance the child seemed healthy. Admission was advised for treatment of a hydrocele of the right testicle. The family history contributed no relevant data.

On inspection, the child was a small male weighing 7 lbs. 7 oz., the skin pale but not icteric, the superficial abdominal veins very prominent, and the abdomen protuberant. He seemed very irritable and cried readily when picked up or disturbed. The rectal temperature was 101.4° F., the pulse and respirations were rapid. The mucous membranes were pale; the nose, throat, and ears not abnormal. The breath sounds were harsh, but no râles and no changes to percussion were noted. The heart was apparently normal. The liver and spleen were both enlarged; the tip of the spleen could be felt almost at the pelvic brim. There was a small umbilical hernia and a right-sided hydrocele the size of a walnut.

Diagnosis: bronchitis and secondary anemia, with associated splenomegaly and hepatomegaly of unknown origin.

The red cell count was 2,400,000, hemoglobin 7.5 gm., platelets 97,000, leukocytes 6100 (20% neutrophils, 77% lymphocytes, and 3% monocytes). Three normoblasts were found, as well as moderate anisocytosis and poikilocytosis. The urine contained a faint trace of albumin and 3 to 8 leukocytes per high-power field. The blood Wassermann test was negative, as were also stool cultures for typhoid and dysentery organisms. A bone marrow smear on the 7th hospital day did not show any abnormal cells. Roentgen ray of the chest revealed no change in the normal appearance of the heart or lungs.

The child had increasing difficulty in breathing and did not take its formula well. Regurgitation after feeding was frequent and several times this was accompanied by attacks of cyanosis. The temperature was never below 100° F. and usually remained about 102° F.; the pulse and respirations were very rapid. After two transfusions of citrated whole blood the hemoglobin rose to 12 gm., the red cell count to 3,660,000 and the leukocytes to 5800 (40% neutrophils, 60% lymphocytes). The number of platelets reached 175,000. At no time did the child show any frank bleeding, but minute petechiæ were observed on the buttocks and chest about 14 days after admission. On the 16th day a splenic puncture was performed and smears made of the aspirated pulp. Histoplasmosis had not been suspected but examination of the smears prepared with the usual Wright stain revealed the typical yeast forms of the fungus in the monocytes (Fig. 1). On the following day another tibial marrow study was made and the same bodies seen in the direct smear. At this time also, another splenic puncture was performed and the marrow and splenic pulp streaked on blood agar plates from which the organisms were subsequently isolated in pure culture. The child died 18 days after admission and 2 days after the diagnosis of histoplasmosis was made, at the age of 9½ weeks.

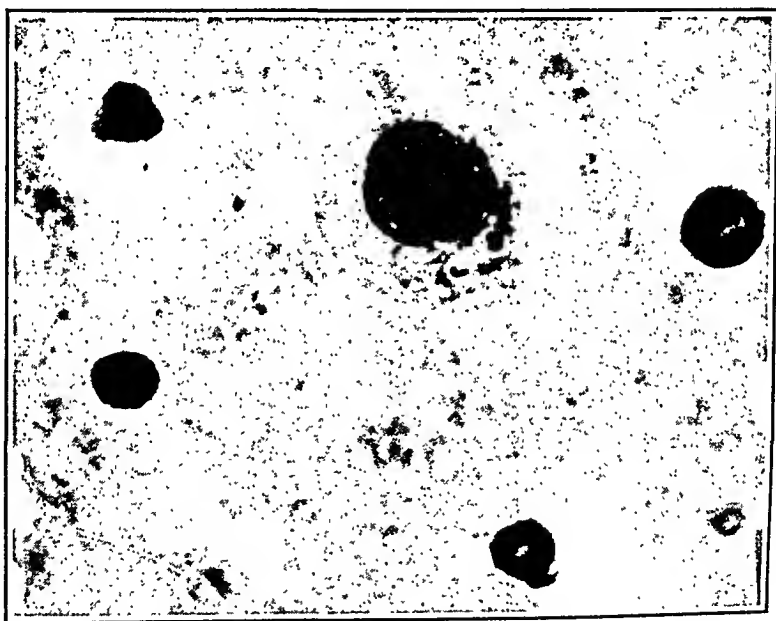


FIG. 1.—Smear of material obtained by splenic puncture. In the center of the field is a monocyte containing two yeast forms of *Histoplasma capsulatum*. (Wright stain.  $\times 1200$ .)

*Autopsy* (1 hour after death). The body weighed 2500 gm. and showed marked evidence of emaciation. The abdomen was distended and large veins were visible beneath the skin. Numerous pale petechiæ were scattered over the trunk; there was no jaundice, cyanosis, or edema. The spleen was palpable almost to the crest of the ilium, the liver extended 2 cm. below the costal margin. Internal examination revealed transparent and glistening pleuræ with no free fluid in either pleural space. The peritoneum was likewise smooth, the stomach and intestine moderately distended with gas. The spleen was visible, occupying much of the left half of the abdomen (Fig. 2). The leaves of the diaphragm had their normal position and the urachus, umbilical veins, and umbilical artery were occluded. The normal visceral relations were maintained and no gross congenital anomalies were present. The pericardium was thin, the serosal surfaces shining; the sac contained 0.5 cc. of clear yellow fluid.

The heart weighed 10 gm., the epicardium was smooth, no petechiæ were present. The myocardium was firm, uniformly red-brown in color. All the chambers were slightly dilated and contained a small amount of cruor. No congenital anomalies were demonstrable. All 4 valves were apparently normal, the papillary muscles well developed, the chordæ tendineæ long and slender. Histologically the myocardium was normal. The coronary vessels and aorta exhibited no abnormalities.



FIG. 2.—The sternum, heart and lungs have been removed, the liver displaced into the chest cavity. At the upper end of the incision a group of enlarged lymph nodes can be recognized; the spleen, though still attached to its pedicle, has been brought into view. The white ruler has a length of 8 cm.

The right lung weighed 13, the left 12 gm. Both organs were pale pink and almost emphysematous in consistency. On section, the upper portion of the right lower lobe contained 4 well-circumscribed, spherical, firm, yellow nodules ranging from 3 to 8 mm. in diameter. The parenchyma surrounding these nodules was essentially normal. No consolidated areas could be demonstrated elsewhere in the lungs, nor could pus be expressed from the bronchioles. The vessels were free of thrombi. Both grossly and under low magnification the nodules resembled those of tuberculous granulation tissue (Fig. 3). There was a large central area of necrosis surrounded by closely packed macrophages nearly all of which contained the yeast form of *Histoplasma capsulatum*. In



the cytoplasm of these cells, as elsewhere in the tissues, the fungus when stained with iron hematoxylin appeared as a spheroidal, refractile body, 2 to 4  $\mu$  in diameter, containing a dark blue-black granule usually 1  $\mu$  in diameter, the whole surrounded by a clear zone, the capsule. Throughout the lung there were focal collections of parasitized monocytes most often grouped about blood-vessels or lymphatics. However, the endothelial cells of the alveolar septa were often similarly involved for some distance from any large vessel (Fig. 4). A patchy monocytic infiltration was also present beneath the visceral pleura.

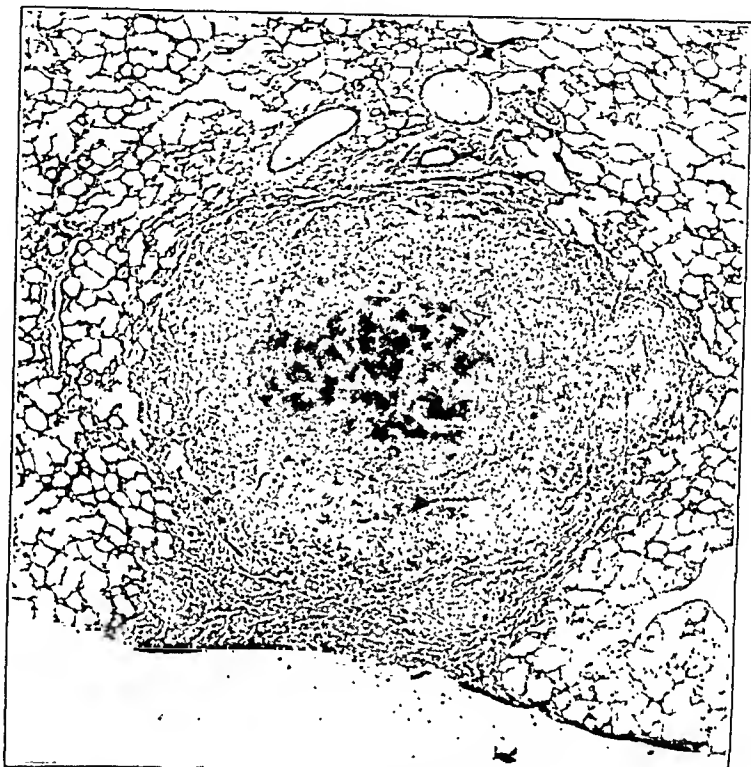


FIG. 3.—A nodule in the lung, illustrating the resemblance of these lesions to a tubercle. A central area of necrosis is surrounded by dense masses of monocytes which have reached the pleura and spread out beneath it. (Iron hematoxylin.  $\times 30$ .) (U. S. Army Medical Museum Neg. No. 74909.)

The spleen weighed 80 gm., had a smooth surface, and a slate-gray capsule free of adhesions. The organ was firm, cut with usual resistance, and had a dark purple pulp that did not scrape away readily on the knife edge. Follicles and trabeculae were indistinct. The mononuclear cells of the Malpighian corpuscles as well as those of the red pulp contained many of the yeast forms. In spite of the widespread involvement of the organ there were no focal areas of necrosis.

Each kidney weighed 20 gm., was of normal shape, size, and consistency. The capsule stripped readily, revealing a smooth pink surface on which the normal fetal lobulations were distinct. On section the cortex was 5 mm. in width, the cortico-medullary markings distinct. The pelvic mucosa of both organs was smooth and white. Microscopically, the kidneys were apparently normal. The mucosa of the ureter and bladder was everywhere white and intact. However, histologic examination revealed small focal collections of monocytes containing the parasite in the submucosa of the urinary bladder. Except for a right-sided hydrocele, the genitalia were apparently normal.

The serosa of the gastro-intestinal tract was everywhere smooth and gray-pink in color. The stomach contained about 6 cc. of partially clotted milk; the mucosa was rugose and free of ulceration. The mucosa of the small intestine showed the normal changes characteristic of the different regions, though Peyer's patches in the ileum were more prominent than usual. No enlargement of the solitary follicles of the large intestine was noted, nor was there blood anywhere in the lumen of the gastro-intestinal tract. The monocytes present in the lymph follicles of the small and large intestine contained many of the yeast forms of the fungus.

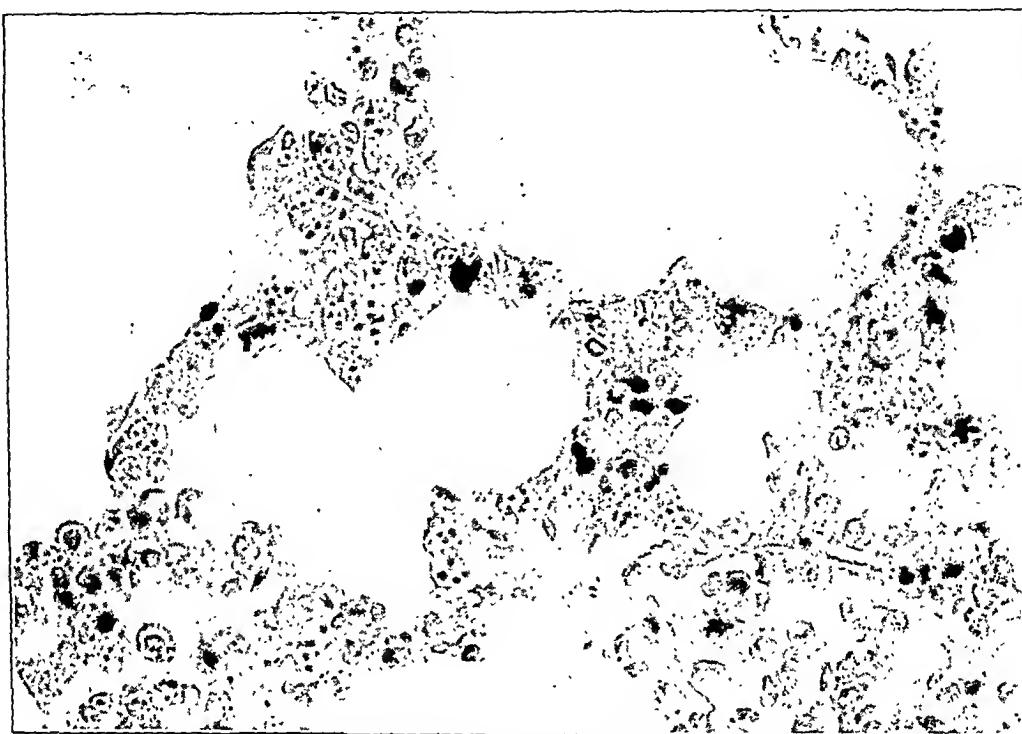


FIG. 4.—A region of the lung some distance from any discrete nodule. The endothelial cells of the capillaries in the alveolar walls contain many of the parasites. (Iron hematoxylin.  $\times 810$ .) (U. S. Army Medical Museum Neg. No. 74904.)

The liver was enlarged (weighed 160 gm.). The surface was smooth, the capsule thin and transparent. On section the parenchyma was a mottled red-brown in color and blood oozed from the severed vessels. The cords of liver cells were separated by a rather loose connective tissue which was also increased in the portal areas. The cytoplasm of the liver cells was granular and only a few contained the parasites; occasional Kupffer cells were crowded with histoplasma. No areas of pseudotubercle formation were present. The small tubular gall bladder had a thin wall, velvety mucosa, and smooth serosa. Dark green viscid bile flowed readily through the ducts and the ampulla of Vater. Histologically the gall bladder was normal.

The pancreas was firm, pink-brown in color, and distinctly lobulated. The surrounding fat was free of necrosis or hemorrhage. No yeast forms were found in the parenchyma, though an immediately adjacent lymph node was heavily parasitized. The adrenals were relatively large, as is normal for an infant of this age. The cortex contained several bright orange granules, the medulla was firm and gray. Microscopically, the cells of the medulla were unexpectedly found to contain many histoplasma, though the cells of the cor-

tex were free. The thymus reached and partly covered the upper portion of the pericardial sac; yet, it was of approximately normal size for the age of the infant. It was soft, dark red and translucent. The lymphocytes were partly replaced by monocytes which in some regions formed focal collections the centers of which showed early necrosis. The monocytes contained many parasites.

The para-aortic lymph nodes in the region of the arch and those surrounding the trachea were matted together in a moderately firm mass. All of these nodes were enlarged and on section were rather opaque, orange-yellow in color. The lymph nodes of the mesentery were likewise enlarged, but translucent and gray-pink. The architecture of the involved nodes was completely destroyed. Solid masses of heavily parasitized monocytes surrounded necrotic areas consisting chiefly of degenerating macrophages (Fig. 5). Though the bone marrow of sternum and femur was bright red in color, histologically hematopoiesis was depressed. The normal marrow cells were replaced by a

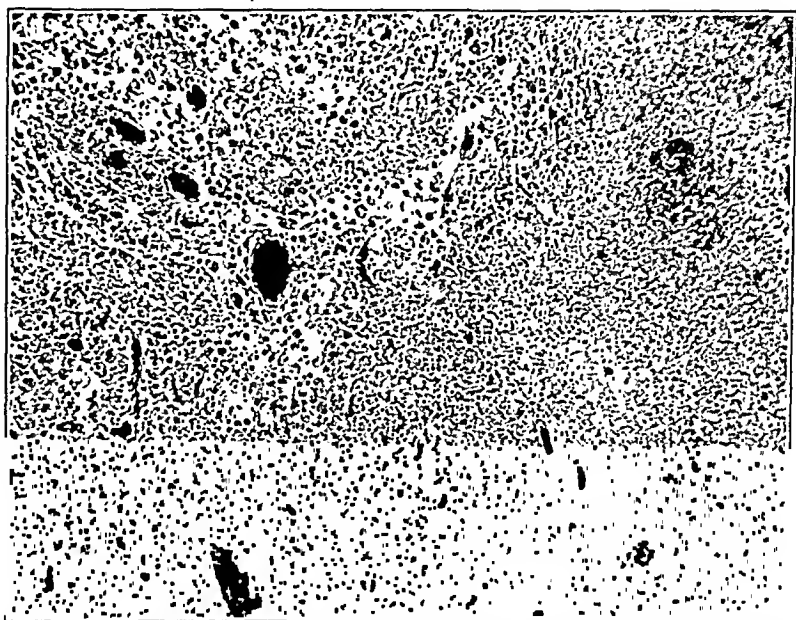


FIG. 5.—Section of lymph node showing a complete loss of normal architecture. On the right the tissue is necrotic, on the left the stroma has been infiltrated by large monocytes which on close inspection are seen to be heavily parasitized. (Iron hematoxylin.  $\times 72$ .)

loose areolar connective tissue in which the blood sinuses were preserved, accounting for the red color on gross examination. Isolated islands of cells contained a few megakaryocytes, myelocytes and erythroblasts, but mature polymorphonuclear leukocytes and plasma cells predominated. No mitotic figures were seen. Occasional monocytes and endothelial cells contained a few yeast forms. In blood smears made at this time the large monocytes were likewise parasitized, though this had not been demonstrable in the circulating blood 2 days before death.

Punctate hemorrhages were present in the falx cerebrum and in the tentorium. No tears, however, were present in any of the cerebral membranes. The gross appearance of the brain was essentially normal, the convolutions complex, the sulci deep. Microscopic examination revealed no abnormal cellular infiltrations. In sections of the skin collections of monocytes containing the histoplasma were found in the loose fatty areolar tissue beneath the corium.

**Cultural Studies.** Material obtained from the spleen and tibial marrow by needle biopsy was streaked on blood agar plates and kept at room temperature. Growth was very slow but after 3 weeks white cotton-like colonies 2 to 3 mm. in diameter began to appear. These were transplanted to Sabouraud's medium where growth was more rapid and the colonies developed their characteristic faint buff color and slightly crinkled margin (Fig. 6). Microscopically they consisted of mycelial threads and prominent spherical chlamydospores. One culture, originally streaked on a blood agar slant and sealed with paraffin, showed minute white colonies scattered over the surface when examined 9½ months later. Microscopically, these consisted solely of the yeast form similar to that found in the tissues. When inoculated on Sabouraud's agar

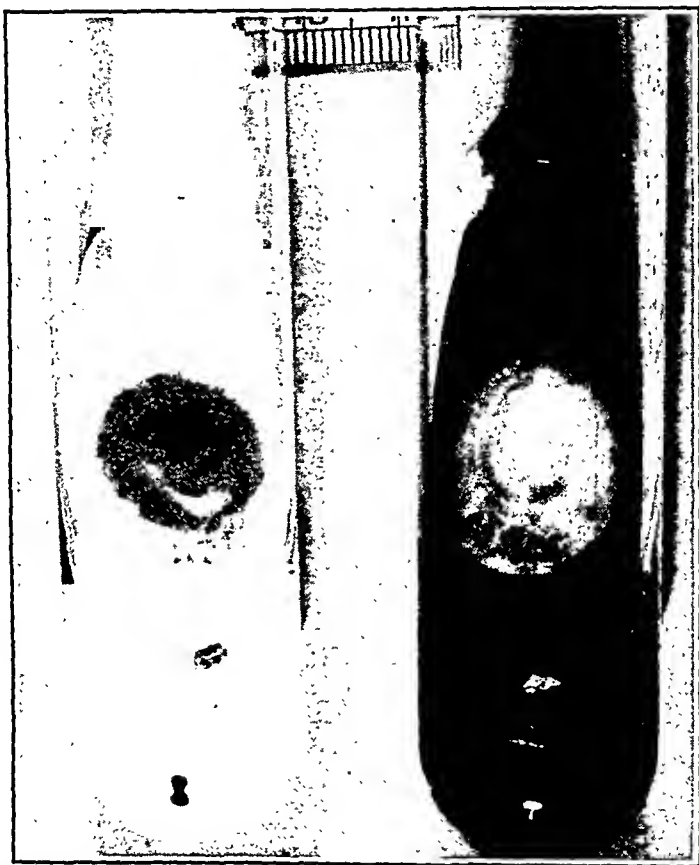


FIG. 6.—Growth of *Histoplasma capsulatum* in the mycelial form on Sabouraud's culture medium. (Natural size.)

these developed into the typical mycelial colonies. Splenic tissue and bone marrow removed at autopsy failed to yield colonies after inoculation on both blood agar and Sabouraud's medium.

**Animal Inoculation.** When the cultures were 6 weeks old, some of them were ground up in sterile saline and injected into the tail veins of 6 mice and the peritoneums of 6 rats. Three mice died after a few days and were negative on examination. The 3 remaining mice died within 3 and 4 months of inoculation. All showed a generalized lymph node involvement characterized by great enlargement and central necrosis. The histologic picture was similar to that found in the human lesion. Outstanding was the enormous size of the liver which in each animal almost filled the abdominal cavity. The surface

was smooth, but scattered over it were many minute yellow patches. Microscopically, the Kupffer cells were found to be almost solely involved. They were greatly enlarged and crowded with 20 to 30 of the yeast forms. The blood channels were almost obstructed and the cords of liver cells had undergone pressure atrophy (Fig. 7). The liver cells themselves were rarely parasitized.

The spleen was enlarged, the follicles and trabeculae could not be recognized. Histologically, the normal architecture had been destroyed by a diffuse dense infiltration of monocytes. These cells together with the endothelial cells of the sinusoids contained large numbers of *Histoplasma capsulatum*. Although

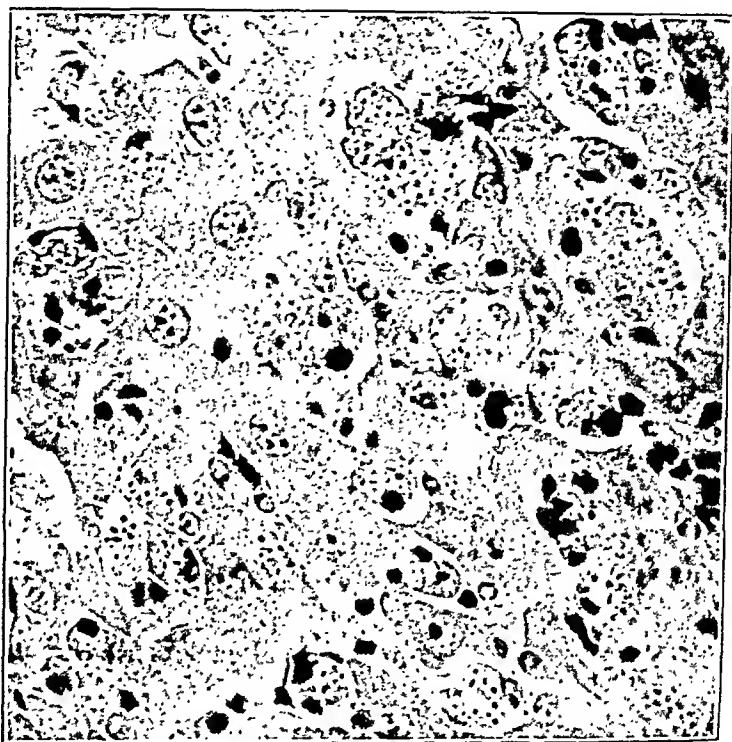


FIG. 7.—Section of liver from a mouse that died 3½ months after inoculation. Each Kupffer cell contains 5 to 30 yeast forms of the fungus and are so enlarged that they almost occlude the blood sinusoids. The cords of liver cells are atrophic. (Iron hematoxylin.  $\times 810$ .) (U. S. Army Medical Museum Neg. No. 74905.)

the kidneys were grossly normal, the endothelial cells of the glomerular capillaries were distended by the parasites and the flow of blood must have been greatly impeded. The endothelial cells of the pulmonary capillaries were likewise heavily parasitized.

After 8 months the rats, which had been inoculated intraperitoneally, were quite healthy. They were sacrificed and on gross as well as microscopic examination failed to reveal any pathologic changes.

**Discussion.** Histoplasmosis is primarily a systemic disease of the lymphoid tissue, characterized by the presence of large numbers of the yeast form of *Histoplasma capsulatum* in the monocytes and endothelial cells of the lymph nodes and spleen. Careful search will usually reveal the parasite in collections of lymphoid tissue in other organs, such as the lungs, intestinal tract, and skin. The parenchymal cells of the

liver and adrenals are frequently involved, and in the case reports now on record nearly every organ has at one time or another been found to harbor the fungus.

At least 5 cases of this disease occurring in infants or young children are recorded, but to our knowledge this is the youngest, death resulting at the age of 9½ weeks. For the first 2 weeks of life the child remained in a large city hospital following which it was at home only a month before being brought to another hospital in the same city. It is probable that the disease was contracted during that month. The infant was never breast-fed, but the source and mode of infection is unknown.

**Summary.** A 7 week old infant was admitted with signs of anemia, splenomegaly and hepatomegaly. The diagnosis of histoplasmosis followed finding the parasite in material obtained by splenic puncture. This was proved by subsequent culture and animal inoculation.

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### THE RELATION OF VITAMIN B TO THE TOXICITY OF PROMIN AS TESTED BY THE SELF-SELECTION METHOD

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PROMIN (sodium p, p'-diaminodiphenylsulfone-N, N'-didextrose sulfonate) exerted certain rather severe toxic influences when it was administered orally to guinea pigs.<sup>6,8,9</sup> These changes included hyperirritability of the animal, severe paralysis of the hind limbs, considerable anorexia and cyanosis. Toxic influences were exerted on the erythrocytes, resulting in their crenation and subsequent removal from the blood stream. As a result, hypochromic anemia, with many of the features of true hemolytic anemia, developed. Abnormal discoloration of the tissues was the result of changes induced in the hemoglobin of the erythrocytes, giving rise to methemoglobin and sulfhemoglobin.<sup>18</sup> These changes were reversible. The bone marrow, as a result of destruction of blood, became hyperplastic and macrocytosis, polychromatophilia and reticulocytosis became characteristic features in the peripheral blood, even in the presence of high concentration of promin in the blood.

In an attempt to study factors which might well alleviate some of the toxic reactions observed therapeutically, the white rat was selected as the animal best suited for further experimental work of the sort contemplated. The rat reacts to promin in ways quite like those of the guinea pig and thus is a most satisfactory test animal. Since certain studies had shown that the toxic effects of some of the sulfon-

amide compounds were mitigated either by the fractions of vitamin B or by diet,<sup>10</sup> I studied the reactions to promin in growing white rats which were fed purified diets, with and without constituents rich in the vitamin B complex. In addition, I studied these toxic reactions in rats which were permitted to imbibe freely the better known fractions of the B complex, provided in suitable water bottles attached to their cages. In a series of studies on the self-selection method, Richter and his colleagues<sup>13-17</sup> described results which indicate the capacities of rats to select adequately the foods, minerals and vitamins required.

This report covers my study of the reactions elicited in white rats by the daily oral administration of promin for 21 days, during which time they were free to help themselves to such fractions of the B-complex as were desired.

**Method of Study.** My initial observations were made on rats housed in small metal cages to which the bottles containing the water-soluble vitamins were attached. I first observed that the rats drank largely from the thiamine bottle after the oral administration of promin. Although the position, on the cage, of the bottle containing the thiamine was changed frequently, I could not help but believe that chance may have operated in selection of the appropriate bottle containing thiamine. Accordingly, a circular maze cage 8 inches (20 cm.) high and 3 feet (91 cm.) in diameter was constructed of galvanized iron. It consisted of a central space 12 inches (30 cm.) in diameter, suitable for the feeding cups, and 3 concentric areas, each 4 inches (10 cm.) wide. Rats passed from the central area to the next adjacent concentric area, and thence to successive concentric areas, through one of two circular openings only slightly larger than the body of the animal. The floor area made of wire mesh, 3 to the inch, comprised 7.1 square feet (6600 sq. cm.). Ten rats were housed in the cage. The water-soluble vitamins were provided in amber bottles attached to the cage at places inconvenient for the rat to approach. In this manner, it would seem that all possibility of chance, in selecting the vitamins, was eliminated. The fractions which were considered most essential were provided in places least accessible.

Male rats weighing from 70 to 85 gm. were selected from our breeding colony. The purified diet used was made up as follows: sucrose, 76 parts; vitamin-free casein, 18 parts; corn oil, 2 parts; commercial salt mixture,\* 4 parts. Cod-liver oil was given frequently during the experiment. Each gram of the diet contained 3.94 calories, of which 77.1% were derived from carbohydrate, 18.3% from protein and 4.6% from fat.

The fractions of vitamin B provided the animals were thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin and choline chloride. These vitamins were graciously supplied by Merck & Co., and I wish to acknowledge my appreciation of their continued interest in my study. The concentrations of these fractions were so arranged in the various bottles that each cc. of solution contained respectively 100  $\mu$ g. of thiamine, 200  $\mu$ g. of riboflavin, 100  $\mu$ g. of pyridoxine, 200  $\mu$ g. of calcium pantothenate, 1 mg. of niacin and 10 mg. of choline chloride. In addition to these 6 bottles, 2 bottles containing distilled water were fixed to the cage. The solutions were made up every 3 days. Volumes in cc. consumed from each bottle were measured daily. Food intake was determined daily.

Rats were placed in the cage for 1 week before promin therapy was instituted. The growth of the animals, their food and vitamin intake were recorded during this initial period. Beginning on the 1st day of the 2d week and for each day thereafter for 3 weeks each rat received 50 mg. of promin by mouth. Weights of the animals were recorded at frequent intervals and their intakes of food and vitamin were recorded daily. Observations were made of the

\* Marketed by the Harris Laboratories, Tukahoe, N. Y.

appearance and behavior of these animals during the period of administration of promin. On the 22d day after the beginning of administration of promin the blood of all animals was sampled by cardiac puncture. Determinations of the concentration of promin in the blood were made by Dr. Osterberg of the Section on Clinical Biochemistry. The morphologic studies on the blood included the total number of erythrocytes and leukocytes per c.mm., the volumes of erythrocytes in cubic microns, the grams of hemoglobin per 100 cc. of blood and the percentage of reticulocytes. All animals were killed by lightly etherizing and exsanguinating them.

The data condensed into this report were assembled from 4 groups of animals: 2 test groups and 2 control groups. In addition to those rats housed in the maze cage, 10 rats were subjected to the same experimental restriction and given promin in the same way but instead of receiving their vitamins *ad libitum* they were given by tube daily 1 cc. of a solution containing 100  $\mu$ g. of thiamine, 200  $\mu$ g. of riboflavin, 100  $\mu$ g. of pyridoxine, 200  $\mu$ g. of calcium pantothenate, 1 mg. of niacin and 10 mg. of choline chloride. The blood of this test group of animals was sampled at the end of the experiment in like manner to that of the other test group of animals partaking freely of the vitamins in the maze cage. Each group of test animals was controlled.

**Results.** 1. *General Appearance and Activity of the Animals.*—Animals fed this purified high carbohydrate diet and given daily by tube the fractions of vitamin B, as indicated, grew satisfactorily, manifested normal hair patterns and did not show any signs of irritability. In the case of animals fed in a similar manner, but given 50 mg. of promin by mouth, definite irritability developed within 24 hours. They became excited and nervous and often jumped from their cages when the door was opened. A number of them chewed their own tails or paws. In a few days hair began to drop off. Some animals presented extreme degrees of alopecia. Severe cyanosis developed. Their food intake was appreciably reduced from 8 to 10 gm. per day to 3 to 5 gm. per day. Their water intake was greatly increased from 12 to 15 cc. per day to 30 to 35 cc. per day. On continued administration of the drug to the animals, tolerance for it appeared to develop. The food intake gradually increased and the nervousness subsided but the extensive alopecia was not relieved.

Animals which were housed in the maze cage and given the same diet and allowed to partake freely of the vitamins grew well and were indistinguishable in appearance from those controls given the vitamins by tube. When promin was given to these animals partaking freely of the vitamins there were no signs of irritability, alopecia did not develop and cyanosis was far less severe than in animals which received the daily allowance of vitamins by tube.

2. *Body Weight.* All animals grew satisfactorily during the initial 1 week trial period before promin was given. The manner of giving the vitamins, by stomach tube as in one group or freely in available bottles as in the other group, did not have any apparent effect on growth during this 1st week. Gains of 18 to 22 gm. per week were recorded (Fig. 1). However, when promin (50 mg. daily by mouth) was given, loss of weight occurred in those animals given the daily constant amounts of the vitamins. On the other hand, those free to select their own, greatly increased their intake of vitamins and did not sustain any loss of body weight. Their growth curve was parallel to,



although not equal to, that of the controls which did not receive promin (Fig. 1). At the end of the experiment (3 weeks of daily administration of promin) the controls had gained an average of

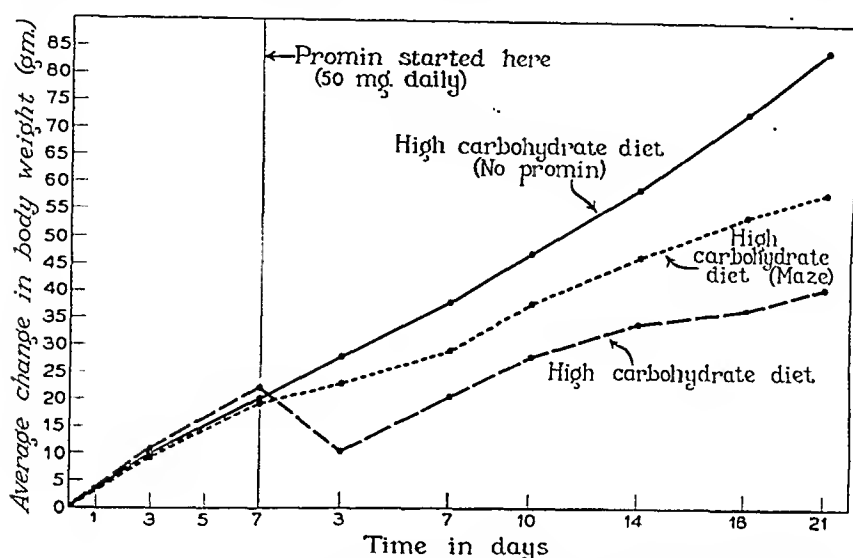


FIG. 1.—Growth of groups of rats fed a purified high carbohydrate diet, with free access to vitamins in the maze or given constant amounts daily by stomach tube. 50 mg. of promin given daily.

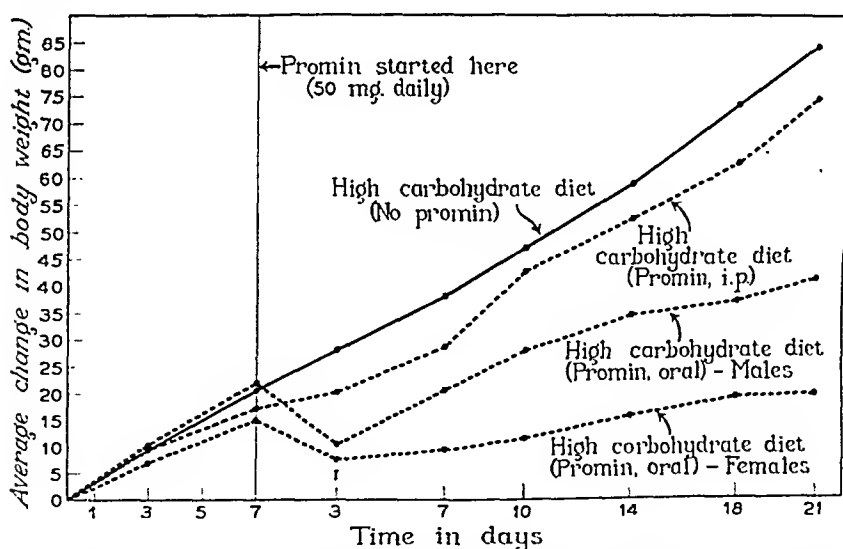


FIG. 2.—Growth of groups of rats fed a purified high carbohydrate diet. Usual vitamin supplements were given to all animals by tube. Growths of females and males when promin was given by mouth, and of males when promin was given intraperitoneally, are shown.

85 gm., those given promin and the vitamins by stomach tube had gained an average of 41 gm. and those given promin with free selection of their vitamins had gained an average of 59 gm.

Female rats, of the same age and weight as males, tolerated the drug less satisfactorily than males and gained but 5 gm. during the 3-week period of administration of promin (Fig. 2). In marked contrast to the toxic effects that oral administration of promin had on growth, I observed that the intraperitoneal administration of the same amounts of the drug for comparable periods had but slight effect on weight (Fig. 2) or appearance of the animal. The characteristic somatic reactions described in an earlier section did not occur in animals given the same amounts of the drug parenterally. It would seem that some of the toxic changes induced by the drug bear some relation to chemical changes which occurred in the gastro-intestinal tract.

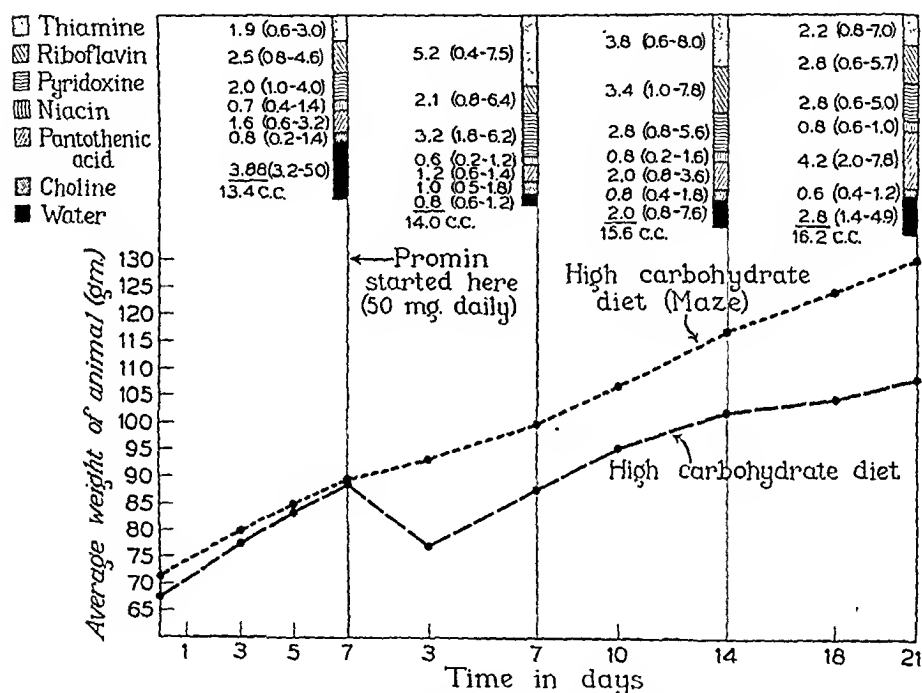


FIG. 3.—Average daily intake in cc. per rat per day of vitamins freely available in bottles attached to the maze. Average intakes per day for 1 week before promin was given, and for 3 weeks of daily administration of promin are shown. The lower lines represent the weights of the rats to which vitamins were freely available in the maze and of those to which constant amounts of vitamins were given.

3. *The Vitamin Consumption per Rat per Day.* The intakes per rat per day of the various vitamins during the week before promin was given, and during the 3 weeks of administration of promin, are shown graphically in Figure 3. These intakes were recorded in cc. Conversion to micrograms is accomplished readily. Although amounts taken were determined daily, only the average daily consumption for each week was plotted. The initial reaction of the animal to promin was to drink larger portions from the bottles containing certain of the vitamins. On some days, an average of 6 to 7 cc. of solutions containing these fractions was taken by each rat. Portions taken from water

bottles, placed conveniently near the food cups, were reduced greatly after administration of promin was started. The amounts of the vitamin preparations taken varied daily. Some days the thiamine intake exceeded that of either riboflavin or pyridoxine. On other days the reverse was true. In any event, either the larger amounts freely taken or the proportions taken by these rats were apparently sufficient to modify the effects of the drug so as to mitigate considerably the toxic changes observed in the rats given the usual daily allowance of these vitamins together with the same amount of promin.

TABLE 1.—SUMMARY OF BLOOD DATA

Group	B-vitamins provided	Amount of promin given	Animals	Erythrocytes (millions per c.mm.)	Volumes in c.micra	Hemoglobin (gm. per 100 cc. of blood)	Reticulocytes (% of erythrocytes)	Blood promin (mg. per 100 cc. of blood)
Control I	By tube	0	10	8.02±0.07	58.1±0.7	13.5±0.2	5.7±0.5	0
Control II	Ad lib. (Maze)	0	10	8.04±0.1	58.4±0.6	12.8±0.2	3.3±0.3	0
Test 1	By tube	50 mg. daily (21 days)	10	3.63±0.3	104.7±2.8	10.3±0.3	58.4±2.3	8.4±0.7
Test 2	Ad lib. (Maze)	50 mg. daily (21 days)	20	5.65±0.1	80.9±1.3	11.4±0.3	25.0±1.7	4.4±0.3

4. *Changes of Some of the Constituents of the Blood.* The data assembled from a study of the blood of the 2 control groups and the 2 test groups of rats are condensed into Table 1. When rats were fed the purified diet and given the usual allowance of vitamins by tube (control Group I), a blood picture developed that was comparable to that of rats of similar age and strain which had been maintained on our standard rat ration. When rats were allowed free access to the vitamins housed in the maze and were fed the same purified diet (control Group II), a blood picture developed which resembled that of control Group I.

When rats were allowed the restricted amount of vitamins (test Group I) and given 50 mg. of the drug daily, pronounced changes developed in the blood. Marked crenation of the erythrocytes, such as was observed to occur in the erythrocytes of guinea pigs given promin, was seen in coverslip preparations of fresh blood from these rats. The blood was very dark. The total erythrocyte count, per c.mm. of blood, ranged from 3,250,000 to 4,800,000. These levels were in marked contrast to the average tabulation of 8,020,000 cells recorded for the control group. Erythrocyte volumes ranged from 87.5 to 126.1 c.micra. Hemoglobin values, in grams per 100 cc. of blood, ranged from 7.6 to 12.4; and the reticulocytes, in percentage of the total number of erythrocytes, ranged from 47.1 to 72.2. The means, together with their probable errors, are set forth in Table 1. The anemia was hypochromic and had some of the features of hemolytic anemia.

The blood sampled from the hearts of rats which lived in the maze cage and had consumed large amounts of the vitamins during the period of administration of promin (test Group II) was considerably better than that in test Group I. There was far less destruction of

erythrocytes. The blood was dark, but less so than in test Group I. Crenated cells in fresh preparations were seen only occasionally. The total number of erythrocytes per c.mm. of blood was 2,000,000 higher and the degree of macrocytosis was correspondingly less. The percentage of reticulocytes, although high, was less than half that encountered in the other test group. The hemoglobin levels, however, were not statistically dissimilar. The report on the promine content of the blood, recorded in mg. per 100 cc. of blood (Table 1), showed that animals, given comparable amounts of the drug by mouth, had a lowered blood level when they partook freely of the vitamins.

**Comment.** These experiments confirm the extensive studies of Richter and his colleagues<sup>13-17</sup> that when rats are given the opportunity for choice, they will select such foods, minerals and vitamins, as prove essential for their growth and physiologic fitness. In this study the selection of the vitamins was made difficult, in that they were placed in positions not easily accessible and remote from food cups; so that the animals had to travel considerable distances to secure these essential nutrients.

Of the 6 vitamins provided, thiamine, riboflavin and pyridoxine were selected most abundantly. The daily intakes of niacin and choline were not increased perceptibly during the 21-day test period. The requirement for pantothenic acid remained unchanged during the first 2 weeks of the test, but during the 3d week the average daily intake of this vitamin significantly increased. It will be noted that the volume of water taken from the water bottles, purposely placed near the food cups, was reduced during the 1st week of administration of promin to a fifth of that taken during the preceding trial week. It is of course obvious that water was taken with all of the water-soluble fractions but it was an interesting fact that these animals passed the water container to obtain solutions of the vitamins. It seems obvious that some appetite for these fractions prompted the search for them.

A survey of the data suggests that, by taking added amounts of thiamine, riboflavin and pyridoxine, the rats of test Group II avoided the immediate loss of weight which occurred when the usual amounts were given. Their growth curve was not quite equal to that of the control group. Further, the extensive patterns of alopecia which characterized prominized rats that received constant amounts of fractions of vitamin B did not develop in rats partaking freely of these 3 vitamins. The extent of cyanosis, however, the dark color of the blood and the enlarged spleens, all characteristic of reactions to promin, were only slightly improved in the animals taking the increased amounts of vitamins. On the other hand, the destruction of blood was less. All data assembled from our study of the erythrocytes, including crenation, total numbers of erythrocytes, cell volumes and percentages of reticulocytes, indicate that by freely taking those vitamins the rats attained a significant improvement of the blood picture.

The apparent thirst or craving for thiamine particularly, indicates perhaps that in the presence of promin a deficiency of thiamine develops. The hyperexcitability and hyperesthesia of these animals, the

posterior paralysis observed in guinea pigs, and the anorexia and ataxia in rats are symptoms which may suggest such a deficiency. The syndrome is somewhat like that described in young foxes wherein spastic paralysis and death were observed to follow, within 48 to 72 hours, the eating of a diet containing uncooked fish. The addition of large amounts of thiamine to the diet prevented the disease and it was concluded that some constituent of raw fish had the capacity to inactivate thiamine.<sup>5,7,22</sup>

A neutralizing power on the bacteriostatic effect of sulfanilamide has been attributed to p-aminobenzoic acid.<sup>20,21</sup> This was explained as due to some competition in the gastro-intestinal tract for essential enzymes. I have observed that yeast will afford some protection against the toxic action of promin. Since yeast is rich in p-aminobenzoic acid, as well as in other vitamins, it may be that some decrease in the synthesis of the essential enzymes may occur in prominized animals.

Changes of the rate of growth of rats given sulfaguanidine or succinyl-sulfathiazole were thought to be due to changes of the production of the essential growth factors in the gastro-intestinal tract.<sup>2,11,12,19</sup> Liver extract, yeast and p-aminobenzoic acid neutralized the effect of sulfaguanidine. Succinyl-sulfathiazole, which changed prothrombin levels as well as reduced growth rates, was antagonized by liver extracts but not by p-aminobenzoic acid.<sup>20</sup>

These drugs produced agranulocytosis, leukopenia and aplasia of bone marrow. They regularly caused lesions of blood-vessels and voluntary muscles, and often lesions of the heart and liver, to develop. The blood dyscrasia was largely prevented by giving whole dried liver or certain liver extracts. Furthermore the dermatitis which developed when either of these two drugs was given may be prevented or successfully treated with crystalline biotin.<sup>1,3,4,19</sup> Studies in this laboratory, as yet unreported, show that whole liver or yeast, but not liver extract, considerably mitigated the toxic changes induced by promin.

**Summary and Conclusions.** A study of the relation of vitamin B to the toxicity of promin, as tested by the self-selection method, is reported. Young male white rats, given orally 50 mg. of promin daily, were allowed to take at will such fractions of the B-complex as they desired. The experiment was continued for 28 days: 7 days without promin and 21 with promin. The conclusions reached were as follows:

1. When rats were allowed to take such vitamins as they desired, the intakes of thiamine, riboflavin and pyridoxine were greatly increased after the oral administration of promin. The amounts taken were often increased sixfold over the amounts considered essential for normal nutrition.

2. In the presence of these added vitamins the syndrome characteristic of reactions to promin did not appear. Hyperesthesia, hyperirritability, anorexia, alopecia and transient loss of weight did not develop.

3. Weight curves parallel to, although not equal to, those plotted for control animals were described for animals taking larger amounts of vitamins.

4. Blood values, including the total number of erythrocytes per c.mm of blood, volumes of erythrocytes and percentages of reticulocytes were all improved significantly in animals which consumed larger amounts of the fractions, over those which received comparable amounts of the drug but were allowed smaller amounts of the vitamins.

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## STUDIES OF THE B VITAMINS IN THE HUMAN SUBJECT

## VII. BLOOD PYRUVATE AND LACTATE-PYRUVATE RATIO FOLLOWING THE INGESTION OF GLUCOSE IN EXPERIMENTAL DEFICIENCY\*

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IN a previous study of experimentally induced deficiency of the B vitamins<sup>5</sup> in a human subject, an abnormal accumulation of blood pyruvic and blood lactic acid was observed late in the deficiency following the ingestion of glucose. The concentration of these substances

\* Sponsored by a fund provided by Mrs. Arthur W. Thompson.

was reduced after the administration of thiamine. Since then Bueding and his associates,<sup>2,3,19</sup> employing a more sensitive pyruvate method, have shown that in patients with clinical evidence of thiamine deficiency a decrease in the rate of removal of pyruvic acid from the blood occurs during the fasting, resting state as well as after the feeding of glucose. More recently Williams *et al.*<sup>17,18</sup> have presented evidence of an impaired breakdown of this acid late in the deficiency of some of their human subjects.

As a result of these observations, a tendency to accept an increased concentration of blood pyruvic acid as diagnostic of early thiamine deficiency has developed. The increase in the pyruvic acid values, however, always occurred in late deficiency, and in the cases of Bueding and associates was associated with clinical conditions where factors other than a vitamin deficiency undoubtedly play a rôle. Before changes in blood pyruvic acid can be accepted as diagnostic of a lack of thiamine, they should be observed consistently in early experimentally induced deficiency, in which other complicating factors which might alter the result, such as variation in physical activity, food consumption and other vitamin deficiencies, can be eliminated.

Accordingly, the present studies were undertaken on 5 women living under carefully controlled conditions of diet and environment. The number of individuals who could be so studied was necessarily limited by the conditions of the experiment. It is our belief, however, that results obtained in this way can be accepted with greater confidence than observations made on a larger number of persons living under less well controlled conditions.

The importance of determining quantitatively both the lactate and the pyruvate was suggested by Stotz and Bessey.<sup>16</sup> They found that in the thiamine deficient pigeon the ratio of lactate to pyruvate, normally constant, was disturbed by an excessively increased concentration of pyruvic acid. Their reports suggested that the simultaneous estimation of both metabolites in the course of a glucose tolerance test might disclose small variations in these substances not detectable by analyses of one or the other separately.

**Methods of Study.** The studies were carried out in a ward of the Thompson Vitamin Clinic\* under the controlled conditions described in an earlier paper in this series<sup>7</sup> where the food, the fluid intake and the physical activity of each subject were rigidly standardized and maintained constant throughout the period of experimental observation. The diet, as described elsewhere,<sup>6</sup> was calculated for each individual so as to contain approximately one-half of the theoretical requirement for thiamine, the other B factors being correspondingly reduced. This type of diet was used because deficiencies of the separate members of the B complex are not likely to be encountered spontaneously in the human being and because the influence of added quantities of other members of the B complex on the utilization of thiamine is not yet well known. The diet supplied adequate quantities of all the other known food factors. Each subject received the diet in constant daily amounts throughout the total period of study. During the initial phase of the experiment the diet was rendered adequate in the B vitamins by a supplement which provided approximately 3 times each individual's minimal requirement for thiamine, riboflavin, niacin,

\* Formerly functioning in the Philadelphia General Hospital, but now closed.

pyridoxine and pantothenic acid.\* This was designated as "Diet A." Following this first period, the supplement was withdrawn and the subjects subsisted on the basal diet alone: "Diet B." Later, thiamine, 35 mg. daily, was added to the diet, which was then termed "Diet C."

The subjects, women without complicating disease or preëxisting deficiency, served voluntarily. All of them had been residents in the Vitamin Ward for a considerable time prior to beginning the present study and also had served as subjects for other studies of deficiency of the B vitamins to be reported elsewhere.

Three subjects (B. A., C. S. and A. M.) were tested after 3 to 5 days on Diet A and again after a short period, 2 to 9 days, on Diet B. None showed any evidence of clinical deficiency at the time of testing. Two subjects (M. B., F. S.) were tested after 1 to 7 days on Diet A and again after 48 to 50 days respectively on Diet B. At the time of testing on this regimen, both subjects showed early clinical evidence of deficiency. Subject F. S. was then tested following 13 days on Diet C at which time the clinical symptoms had improved.

The chemical methods have been presented previously by one of us.<sup>11,12</sup> Lactic acid analyses were done by the Barker-Summerson modification<sup>1</sup> of the Miller-Muntz method.<sup>14</sup> Blood pyruvate and lactate levels were determined with the patients fasting and at rest and also following the oral administration of glucose, according to the procedure already described.<sup>12</sup>

**Results and Discussion.** In the control experiments (Diet A) all subjects showed normal pyruvate levels (Table 1) under basal conditions and temporary increases following the feeding of glucose. The response of pyruvate was similar to that previously described in normal subjects.<sup>11</sup>

TABLE 1.—BLOOD PYRUVIC ACID, FASTING AND FOLLOWING THE INGESTION OF GLUCOSE ON AN ADEQUATE DIETARY REGIMEN (DIET A)

Subject	Time on diet (days)	Time after ingestion of glucose				
		Fasting	0.5 hr.	1 hr.	2 hrs.	3 hrs.
		Mg. pyruvic acid per 100 cc. blood				
F. S.	1	0.77	0.87	0.98	0.99	0.77
C. S.	3	0.59	0.66	0.74	0.83	0.70
M. B.	7	0.75	0.76	1.12	1.13	0.84
Average:		0.70	0.83	0.95	0.98	0.77

TABLE 2.—BLOOD PYRUVIC ACID, FASTING AND FOLLOWING THE INGESTION OF GLUCOSE WHILE TAKING A DIET DEFICIENT IN THE B VITAMINS (DIET B) FOR A TIME INSUFFICIENT TO PRODUCE CLINICAL MANIFESTATIONS OF DEFICIENCY

Subject	Time on diet (days)	Time after ingestion of glucose				
		Fasting	0.5 hr.	1 hr.	2 hrs.	3 hrs.
		Mg. pyruvic acid per 100 cc. blood				
B. A.	2	0.84	0.84	1.17	1.08	0.96
C. S.	7	0.95	1.39	1.28	1.17	0.96
A. M.	9	1.07	1.16	1.53	1.62	1.03
Average:		0.95	1.13	1.33	1.25	0.98

When the subjects had consumed the basal diet (Diet B) for a brief period, insufficient to produce clinical manifestations of thiamine deficiency, some increase in the height of the pyruvic acid curve occurred following the ingestion of glucose (Table 2). This was due to

\* Kindly supplied as "Pentaplex" by Smith, Kline & French Laboratories, Philadelphia.



the high initial as well as subsequent pyruvate values for both Subjects C. S. and A. M. However, the shape of the curve in each instance was not abnormal and values returned to the fasting level at the end of 3 hours. Thus, as would be expected, no change in rate of removal of pyruvic acid was detectable after 2 to 9 days on the deficient diet.

TABLE 3.—DAILY INTAKE OF THIAMINE (DIET B) AND AVERAGE DAILY URINARY EXCRETION OF THIAMINE AT TIME OF TEST

Subject	Days on Diet B	Daily intake of thiamine ( $\mu$ g.)	Average daily urinary thiamine ( $\mu$ g.)
B. A. . . . .	2	274	67
C. S. . . . .	7	262	61
A. M. . . . .	9	262	
M. B. . . . .	48	279	12
F. S. . . . .	50	214	11

After 48 to 50 days on the basal diet (Diet B) definite evidence of deficiency was present in subjects M. B. and F. S. Both showed the typical<sup>4,18</sup> early symptoms of increased irritability and apprehension, together with gastro-intestinal complaints and shifting pains particularly in the lower extremities. Average daily urinary excretion of thiamine was commensurate with intake (Table 3) and cannot, as discussed elsewhere,<sup>7</sup> be regarded as a measure of the degree of deficiency. In both these subjects, however, a delayed excretion of a test dose of thiamine occurred, a response that correlates fairly well with the onset of clinical deficiency.<sup>9</sup> In both instances the manifestations of deficiency were those which, unless the subjects had been under careful supervision and unless it had been known that deficiency of the B vitamins was the only operating factor, might have been missed. The deficiency, therefore, was of the "sub-clinical type" for which a diagnostic test is eagerly sought. The pyruvic acid values in these subjects (Table 4) did not differ in any significant way from the normal or from those after a short period on the basal diet. The fasting values were within normal limits, and the shape of the curves after glucose was, likewise, entirely normal. The 3d hour value in subject M. B. was slightly elevated but not to a degree to be regarded as significant. Administration of thiamine (35 mg.) for 13 days to subject F. S. produced no change in pyruvic acid values although clinically she was much improved.\*

It is greatly regretted that owing to the exigencies of the war, it was impossible to prolong the experiment further, in order to see at what point in the development of deficiency, abnormal values would have first become evident. From our own experience<sup>5</sup> and that of Williams and his associates,<sup>18</sup> however, it seems fairly certain that such changes develop only when clinical deficiency is well established. In view of these facts it is most unlikely that alterations in blood pyruvic acid, either fasting or after the administration of glucose, can serve as a diagnostic test for early deficiency. Furthermore, given abnormal values for pyruvic acid, great caution should be used in attributing

\* Blood pyruvic acid values were: fasting, 0.73;  $\frac{1}{2}$  hour, 0.86; 1 hour, 1.17; 2 hours, 0.94; 3 hours, 0.81.

such changes to deficiency of the B vitamins in the absence of definite clinical evidence of deficiency since many other factors<sup>8,13</sup> are known to alter blood pyruvic acid concentration. Blood lactic acid values paralleled the pyruvic acid, resulting in a constant lactate-pyruvate ratio (Table 5). Thus, determination of this ratio added nothing to the sensitivity of the test as observed in thiamine deficient pigeons.<sup>16</sup>

TABLE 4.—BLOOD PYRUVIC ACID, FASTING AND FOLLOWING THE INGESTION OF GLUCOSE WHILE TAKING A DIET DEFICIENT IN THE B VITAMINS (DIET B) FOR A TIME SUFFICIENT TO PRODUCE EARLY SYMPTOMS OF DEFICIENCY

Subject	Time on diet (days)	Time after ingestion of glucose				
		Fasting	0.5 hr.	1 hr.	2 hrs.	3 hrs.
		Mg. pyruvic acid per 100 cc. blood				
M. B.	48	0.87	1.04	1.16	1.03	1.07
F. S.	50	0.91	0.98	1.19	1.33	0.99
Average:		0.89	1.01	1.18	1.18	1.03

TABLE 5.—BLOOD LACTATE-PYRUVATE RATIO, FASTING AND FOLLOWING THE INGESTION OF GLUCOSE IN THE DIFFERENT EXPERIMENTAL PERIODS

Subject	Time after ingestion of glucose				
	Fasting	0.5 hr.	1 hr.	2 hrs.	3 hrs.
Diet A:					
F. S. . . . .	7.5	7.7	9.4	9.6	8.6
C. S. . . . .	10.2	10.2	10.1	10.0	9.3
M. B. . . . .	9.9	9.6	9.0	9.4	10.4
Diet B (2-9 days):					
B. A. . . . .	9.0	9.5	9.8	10.0	10.6
C. S. . . . .	8.0	9.3	9.8	8.5	8.0
A. M. . . . .	8.2	8.9	9.6	9.6	9.5
Diet B (48-50 days):					
M. B. . . . .	8.4	9.1	9.2	11.1	10.7
F. S. . . . .	7.9	8.3	9.4	9.9	8.7
Diet C:					
F. S. . . . .	8.8	9.3	9.7	10.7	9.1

**Summary.** Blood pyruvic acid values as well as the blood lactate to pyruvate ratio, fasting and following the administration of glucose, did not differ from the normal in 5 subjects living under carefully controlled conditions and ingesting a diet deficient only in the B vitamins, even though manifestations of "sub-clinical" deficiency became evident in 2 of them.

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# PROGRESS OF MEDICAL SCIENCE

## SURGERY

UNDER THE CHARGE OF

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### THE RÔLE OF NUTRITION IN PREOPERATIVE AND POSTOPERATIVE CARE

#### A REVIEW

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ALTHOUGH treatises on therapeutics refer many times to increasing the nutritional state and general resistance of patients, the true significance of this often repeated phrase is probably not realized by many physicians and surgeons. No one need impress upon the medical profession the importance of nutrition; it is evident in everyday practice. We need to know more about methods of reestablishing and maintaining the nutritional status of patients. The science of nutrition has progressed rapidly in the past 25 years. It has been said that practicing physicians have not kept pace with the increased knowledge of nutrition.

That nutritional deficiencies do decrease resistance to generalized infections has been demonstrated by E. C. Robertson.<sup>43</sup> She has shown that deficiencies of thiamin chloride, the B complex, vitamin D, vitamin C, protein or minerals reduce the ability of rats to survive a generalized infection. Undoubtedly, further investigation will augment this list. It is apparent that the diets previously regarded as adequate do not offer an optimum amount of nutritional elements. This fact is attested by the recent encouragement of bakers by The Food and Drug Administration to enrich baking products with added vitamins. Campbell and Sherman<sup>10</sup> have shown by animal experiments that, starting with a diet already considered adequate, the feeding of additional food elements will increase the rate of growth, will produce a larger adult size with earlier maturity, and will prolong the period of adult life.

A fundamental understanding of the problems of nutrition and an application of that knowledge will reduce operative morbidity and mortality. Nutritional problems in surgery are numerous. It is not possible to dis-

\* Now on Active Service in the Armed Forces.

cuss in a few pages all of these problems. In the following paragraphs the items of nutrition which most concern the surgeon are briefly discussed in the light of their influence upon surgical patients. Included also are limited discussions of the nutritional treatment of some specific surgical conditions.

**Protein.** Protein is necessary for growth and repair of tissues, for formation of secretions, both internal and external, for the transport of fat, and at times for the production of energy. The reserve store of protein in the adult body averages 4.5 pounds.<sup>8a</sup> In the hydrated state this protein may represent as much as 26 pounds, a considerable portion of the body weight. If this protein were the only store called upon to supply energy during a period of starvation, these 26 pounds of hydrated protein could supply the basal caloric requirements of the average adult for only 5 days. Most common chronic protein deficiencies are caused by (1) inability to ingest food due to pain or vomiting, (2) inability to assimilate food due to intrinsic gut changes, (3) a diet prescribed by one unfamiliar with the problems of nutrition, and (4) inability to obtain food for economic reasons. The body stores of protein are reflected in the level of the serum protein. The serum protein is, therefore, our measuring stick of the body store of protein. Obviously, in acute hypoproteinemia, as caused by burns, hemorrhage, marked intestinal distention, and intestinal obstruction, the serum protein level is not an accurate index of the body reserve of protein.

Hypoproteinemia causes edema, poor wound healing, retarded bone healing, a decreased resistance to generalized infection, a delay in gastrointestinal motility, and it may be the cause of failure of function of surgical gastro-intestinal stomata. The surgeon should be greatly concerned when his patient has a low serum protein level.

Clinical edema, which represents an increase of at least 10% of weight by edema fluid, usually appears when the serum protein level falls below 5.5 gm. %; however, *incipient edema* appears as soon as the serum protein level falls below 7 gm. %. One should not delay the treatment of hypoproteinemia until clinical edema appears, for, long before this stage of edema is reached, the adverse effects of hypoproteinemia and *incipient edema* are at work.

Thompson, Ravdin and Frank<sup>50</sup> were the first to demonstrate that hypoproteinemia caused wound dehiscence. Hartzell and Winfield<sup>18</sup> found in a study of wound separation that all of the patients had a deficiency of protein or vitamin C or both. The retardation of the healing of fractures in the presence of hypoproteinemia was noted by Rhoads.<sup>41</sup> Following the suggestion of Jones and Eaton,<sup>23</sup> Barden, Ravdin and Frazier<sup>4</sup> first showed that hypoproteinemia could cause a failure of function of an otherwise mechanically perfect gastro-enterostomy stoma. Delay in gastric emptying and the delay in the stomach-to-cecum time in the presence of hypoproteinemia, and the return of gastro-intestinal motility to normal following the restoration of the serum protein to normal levels have been reported by Mccray, Barden and Ravdin<sup>25</sup> and Barden *et al.*<sup>5</sup>

Acute hypoproteinemia is overcome by immediate replacements of large quantities of blood and plasma. The magnitude of the problem of treating chronic hypoproteinemia becomes apparent when one realizes that according to Elman's calculations<sup>12</sup> 375 gm. of protein are required each day for a period of 10 days in order to elevate a serum protein level from 5 to 7 gm. %. Sachar and Elman<sup>44</sup> have found that for every gram of protein deposited in the serum 30 gm. are deposited in the body in general.

Obviously, the easiest and best way of overcoming hypoproteinemia is by means of a high protein, high caloric diet. Such a diet might well be recommended preoperatively just as soon as a hospital appointment is made for an elective operation. Patients who cannot ingest or assimilate food must receive protein by some other route. Ravdin has demonstrated the effectiveness of feedings by means of a gastric or jejunal tube.<sup>46</sup> Plasma has been shown by Whipple<sup>53</sup> to be the most efficiently utilized protein in the treatment of hypoproteinemia. Protein hydrolysates may be administered by gastric or jejunal tube<sup>46</sup> and occasionally by the rectal route. Mixtures of amino acids are now being prepared commercially for intravenous use. It is often possible to maintain a normal adult in nitrogen balance with these preparations. Although rapid strides are being made in the preparation of amino acids, it has not yet been possible to maintain chronically ill patients in nitrogen balance.<sup>29</sup>

The Miller-Abbott tube<sup>1</sup> has made it possible to restore nutrition prior to operation in the presence of partial or complete-intestinal obstruction. A modification of the Miller-Abbott tube, the Abbott-Rawson tube,<sup>2</sup> has made it possible to maintain nutrition in the immediate postoperative period following operations of the stomach and duodenum. The advantages of maintaining nutrition during this period have been emphasized by Ravdin, Stengel, Prushankin,<sup>38</sup> and Mulholland.<sup>29</sup> If an Abbott-Rawson tube has not been used it is occasionally necessary to perform a jejunostomy in order to restore the nutritional status of certain patients.

**Carbohydrate.** Carbohydrate and fat together primarily furnish energy to the body. Carbohydrate has a protein-sparing action. During World War I large groups of people in Central Europe who received insufficient food were found to have anemia and edema secondary to hypoproteinemia. Yet it was observed that when an adequate amount of carbohydrate was added to their diet the protein deficiency symptoms disappeared entirely.<sup>34</sup> When present in amounts in excess of the caloric requirements, carbohydrate may be stored as fat.<sup>35</sup> Carbohydrate is of the utmost importance to the surgeon because it is easily prepared and administered by the intravenous route as glucose solution during the periods when a patient is unable to take nourishment by mouth. Glucose, when administered intravenously, is 95 to 98 % utilized according to Winslow.<sup>54</sup>

**Fat.** Fats yield almost twice as much energy in the form of heat as does carbohydrate. Fat alone does not have the same protein-sparing action that carbohydrate exhibits, but almost any mixture of fat and carbohydrate will spare protein to the same extent as carbohydrate alone. Myers and Blumberg,<sup>30</sup> Narat,<sup>31</sup> and others have prepared emulsified fats for intravenous use. An excessive store of fat increases operative mortality. Fatty tissues heal poorly and are prone to develop hematomata, serum collections, and infections.<sup>52</sup> Fat has indirectly an adverse effect upon the liver. A high liver lipid content predisposes to liver injury, as has been shown in the experimental animal.<sup>16</sup>

**Fluids.** Water is an important nutritional item. Man cannot exist for long without it. Adequate hydration and its relation to surgery was recognized many years ago. Maintenance of the body fluid level, both intracellular and extracellular, is of the utmost importance to the surgical patient. Recent literature contains many excellent articles on the subject of fluid balance.<sup>11,25</sup> Fluid intake should be so adjusted that the urinary output is maintained between 1000 and 1500 cc. daily.

When clinical signs of dehydration are present, Maddock and Collier<sup>26</sup> have shown that there is dehydration equivalent to at least 6 % of the

body weight. A dehydrated patient should receive 6% of the body weight in fluid in addition to his normal fluid requirement. In addition to its other effects, dehydration also retards wound healing.<sup>9</sup>

**Sodium Chloride.** Along with the understanding of fluid balance we have gained a knowledge of the importance of electrolyte balance. Water balance cannot be separated from salt metabolism because the retention of water is largely dependent upon the amount of sodium in the body tissues. In order to compensate for salt losses of the skin and feces, to facilitate acid base adjustments, and to maintain the body fluid volume, the daily salt intake should be from 5 to 10 gm.<sup>6</sup> Any excessive loss of electrolyte must be replaced with additional salt and fluid. Marked lethargy, dulling of sense, taste, and mentality, muscular twitching, loss of elasticity of the skin, acidosis or alkalosis are signs of hyponatremia. Calculated doses of sodium chloride as suggested by Power *et al.*<sup>36</sup> are used to overcome chloride deficits.

**Vitamins in Surgery.** To produce preoperatively and postoperatively a more nearly normal physiologic state in the surgical patient, the administration of vitamins as advocated by Vorhaus<sup>51</sup> is often essential. Vitamins are essential for normal physiologic processes in the body. A deficiency of vitamins may cause serious physiologic disturbances long before the gross pathologic changes characteristic of avitaminoses become apparent.<sup>47</sup> The members of the B complex act as enzymes and co-enzymes for carbohydrate metabolism in the body and as such they are being constantly destroyed.<sup>45,35,49</sup> Sydenstricker<sup>47</sup> has noted that a critically ill patient, receiving intravenous glucose as his only caloric intake, may develop signs of thiamin chloride deficiency in as short a time as 3 or 4 days. Similarly a nicotinic acid deficiency may occur with the development of glossitis and mental confusion. Symptoms of both thiamin and nicotinic acid deficiency disappear with the administration of these vitamins according to Sydenstricker.<sup>47</sup> Frazier and Ravdin<sup>43</sup> have noted that hyperthyroid patients following the administration of thiamin, show greater increase in weight and appetite, and a greater fall in heart rate than patients who received no additional vitamins.

**Vitamin A.** Vitamin A itself probably does not materially affect the course of surgical patients. That vitamin A is of value in reducing the incidence of respiratory infections and that it is of value in the stimulation of the healing of surgical wounds has not been generally accepted.<sup>45</sup>

**Vitamin C.** It has been demonstrated by many investigators that vitamin C is essential to normal wound healing.<sup>19,48,55</sup> Vitamin C is necessary for maturation of procollagen to the collagen of fibrous tissue. From 27 to 44 % of apparently normal people have vitamin C plasma levels below the lower limit of the normal accepted vitamin C range.<sup>20,42</sup> Patients with certain chronic surgical diseases have, on the average, vitamin C levels which are well below the lower normal limit of plasma vitamin C level.<sup>7,8,21,56</sup> A definite fall in the plasma vitamin C level following operative procedures has been noted by Bartlett *et al.*<sup>7</sup>

**Vitamin D.** The fat-soluble vitamin D is important in the body metabolism of calcium and phosphorus. In its absence more calcium is lost from the body than is furnished by the diet. Vitamin D is necessary for normal bone growth and bone repair.

**Vitamin K.** Vitamin K is a group of specific substances associated with the maintenance of a normal blood clotting mechanism. Deficiencies predispose to hemorrhage. It is now possible to restore the blood prothrombin time to normal in the majority of patients within 72 hours. The

comparative activities of the more commonly used antihemorrhagic compounds have been listed by Starr.<sup>45</sup> Treatment of resistant cases of prothrombin deficiency has been adequately discussed by Rhoads.<sup>40</sup> Destruction of prothrombin occurs constantly in the body<sup>3</sup> and, therefore, when the pathologic state which caused the deficiency remains unchanged, repeated intravenous injections or oral administration (together with bile salts) is necessary until the clinical condition improves.

**Diet in Biliary Tract Diseases.** The preoperative preparation of patients with liver disease is of the utmost importance, especially in those patients with a history of prolonged or intermittent jaundice. The danger in biliary tract surgery lies mainly in the fact that operation adds further damage to an already impaired liver.

Opie and Alford<sup>32</sup> in 1914 reported on the beneficial effect of a high carbohydrate diet in protecting the liver from chloroform necrosis. For a number of years following their report, carbohydrate by mouth and by vein was used to treat liver diseases and to protect the liver from further damage. More recently Goldschmidt, Vars and Ravdin<sup>16</sup> have shown that the degree of liver injury is dependent upon the amount of fat present in the liver, no correlation being noted between the level of liver glycogen and the degree of liver damage. A diet containing 20% of protein, 75% of carbohydrate and 5% of fat has proved its superiority over other types of commonly used diets in preparing the liver for minimal damage by decreasing the amount of liver fat in the experience of Ravdin *et al.*<sup>39</sup> and Johnson *et al.*<sup>22</sup>

**Nutrition in Gastric and Duodenal Surgery.** Probably no major surgical procedure requires as meticulous and thorough preparation and aftercare as do operations of the stomach and duodenum. Factors to be considered are (1) fluid requirements, (2) electrolytic requirements, (3) caloric intake, (4) vitamin intake, (5) protein intake, and (6) the mechanical difficulties. All but one of these 6 factors are problems of nutrition. Care of these patients has been described, among others, by Ravdin,<sup>37</sup> R. Graham,<sup>17</sup> and Kiefer.<sup>34</sup> The advent of the Abbott-Rawson tube<sup>2</sup> has greatly facilitated the postoperative treatment of these patients. Exteriorization of the Abbott-Rawson tube through the abdominal wall, as suggested by Bisgard,<sup>9a</sup> appears to have advantages. Failure of function of a newly formed stoma may be due to the edema which accompanies hypoproteinemia, as has been pointed out by Meeray, Barden and Ravdin.<sup>28</sup> When feedings are begun by mouth, fatty foods are to be avoided, because Pendergrass *et al.*<sup>33</sup> have demonstrated a delay in gastric emptying time following ingestion of fat.

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## OPHTHALMOLOGY

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## SENILE CHANGES IN THE CHOROID AND RETINA

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A REVIEW of the so-called senile degenerations of the choroid and retina might be considered to be of no more than academic interest if one adopts the rather hopeless attitude suggested in Zentmayer's<sup>22</sup> statement: "There are of course many other senile changes met with in the eye such as the macular degenerations, but their early detection is not of great importance, as we can do nothing for them." Crisp<sup>6</sup> states: "Senility and death begin at birth, or rather in the early stages of embryonic development. The vascular system, which has so obvious a share in the aging process, is constantly in course of modification, and each step in such modification may logically be considered as a move in the inevitable

march from conception toward death." However, Crisp<sup>6</sup> recognizes the "lack of unanimity in disintegration" in the human organism and states that "the tissues of the eye illustrate beautifully this tendency to unequal aging as between different parts of the body" and that "the extent to which changes commonly regarded as senile are really due to infection or other extraneous influence may often be in doubt." Along these lines, de Schweinitz,<sup>17</sup> in 1925, called attention to the favorable influence upon the early stages of so-called senile macular retinochoroiditis of the elimination of foci of infection, notably in the prostate, bladder and teeth. And even Zentmayer<sup>22</sup> himself stated that "indulgences which in early life are without serious or appreciable injury to the body tissues and functions, in later life either as a result of cumulative action of the toxic agents or of lowered resistance of the tissues, or both, may bring in their train functional impairment or serious organic change." More recently experts in nutrition and nutritional diseases have called attention to the similarity or identity between certain lesions of the eye commonly considered to be "senile" and those due to chronic long-continued avitaminosis or nutritional deficiencies existing from birth or even before birth in the mother. This attitude to the mode of development or rather prevention of at least some of the "senile" changes in the eye is epitomized in the statement of Dr. Katherine Bain<sup>2</sup> that, in order to have well-nourished adults in 1961, babies must be fed properly now. With these ideas in mind, it was thought that a review of some of the more common so-called senile lesions in the retina and choroid and of their histologic background might stimulate their earlier recognition in the chronologically presenile group of individuals in whom they occur not infrequently and in whom perhaps a causative background other than age alone might be more readily discovered than in the truly aged.

In the choroid, according to Duke-Elder,<sup>7</sup> the essential senile changes occur primarily in the blood-vessels and secondarily in the tissues dependent on the choroid for nourishment, the lamina vitrea of the choroid, the pigment epithelium of the retina, and in the retina itself, especially in the macular region. It is rather difficult to determine, at least ophthalmoscopically, the types or instances of choroidal arteriosclerosis which may be said to be dependent on age alone. Histologically, 3 types of choroidal arteriosclerosis may be distinguished. Duke-Elder<sup>7</sup> states that in the senile atherosclerotic type of choroidal arteriosclerosis there is "a deposition of lipoids in the intima associated with fibrous tissue proliferation and followed by hyaline degeneration which may be extensive, sometimes with the formation of large atheromatous plaques. In this senile change the thickening and hyalinization of the adventitia and media may be so marked that it may be difficult to differentiate these structures from the hyalinized connective tissue surrounding the vessels; but there is little narrowing of the lumen or tendency to obliteration." However, he states later that the lumen may be restricted or even obliterated by the hyalinization of the walls "so that the vessel takes on the appearance of a white opaque tube with thick striated walls." There may be an accompanying thickening of the walls of the veins by connective tissue increase. It is possible that the vessels with obliterated lumens belong more strictly to the lesions associated with hypertensive disease which Duke-Elder<sup>7</sup> refers to as the "more arteriosclerotic types" in which "there is connective tissue proliferation of the media with reduplication of the internal elastic lamina so that eventually extreme narrowing or even obliteration of the lumen may follow. The ultimate result may be complete occlusion of

the vessel with advanced hyaline or lipid degeneration and complete degeneration and disorganization of the cells of its walls." It is probable that endothelial proliferation plays a rôle also in the obliteration of the lumen of the choroidal arteries in hypertensive disease. A third histologic type of choroidal arteriosclerosis is described by Cohen<sup>5</sup> as a replacement of the muscular coat of the arteries by fibrous acellular tissue with some degeneration of the elastic lamina but without actual thickening of the walls or narrowing of the lumen.

From the clinical ophthalmoscopic standpoint it seems possible that the hypertensive or "arteriosclerotic" type is represented by the isolated, apparently obliterated or essentially obliterated choroidal arteries associated with "Siegrist's dots." The clinical interpretation of the scattered patches of choroidal arteriosclerosis seen mainly in elderly individuals but not associated with loss of vision would be much clearer if it was known definitely whether the sclerosis was histologically of the arteriosclerotic or the atherosclerotic type. Cohen's findings suggest that the so-called primary choroidal sclerosis, the principal symptom of which is loss of vision due to secondary degenerative changes in the retina, may have as its histologic basis the fibrosis of the media that he describes. However, on the basis of the histologic findings it is rather difficult to explain the ophthalmoscopic appearance of vascular obliteration to which both Cohen<sup>5</sup> and Duke-Elder<sup>7</sup> refer. The principal types of primary choroidal arteriosclerosis which are observed are the diffuse choroidal sclerosis or atrophy of the choroid (Morton),<sup>13a</sup> the central areolar choroidal atrophy, and the massive peripapillary choroidal sclerosis which is probably an exaggeration or extension of the so-called senile halo. It is of interest that all these types of choroidal sclerosis and atrophy, while they occur most commonly in individuals over 60 years of age, are seen occasionally in the young, and that there seems to be a certain familial incidence.

Considerable confusion exists concerning the clinical interpretative value of choroidal arteriosclerosis with reference to the presence of arteriosclerosis elsewhere in the body, particularly in the brain and in the coronary arteries. By some authorities, choroidal arteriosclerosis is regarded as a constant age change. Thus, Kerschhaumer<sup>12</sup> stated that it was present in 50 % of people between the years of 40 and 50, in 75 % of those between 50 and 60, and in all over 60 years of age. And Friedenwald<sup>8</sup> concluded that arteriosclerosis in the choroid starts usually at the age of 40 and, as in the spleen, progresses slowly without any direct relationship to general vascular disease. It is one's impression, however, that, though this may be true from the histologic standpoint, yet, from the ophthalmoscopic standpoint, more careful differentiation of the types of visible choroidal arteriosclerosis may yield ultimately some information of value with regard to the diagnosis of arteriosclerosis in other parts of the vascular system.

The visibility of changes in the larger vessels of the choroid depends to a certain extent upon the amount of pigment present normally in the individual choroid and retinal pigment epithelium. Thus, early changes are seen most readily in lightly pigmented eyes. As the sclerosis becomes more advanced, however, secondary changes in the intervacular stroma (degeneration), in the choriocapillaris (sclerosis and atrophy), and in the retinal pigment epithelium (degeneration, depigmentation and atrophy) increase the visibility of the larger choroidal vessels even in the normally heavily pigmented eye. These secondary degenerative changes in the choriocapillaris and in the retinal pigment epithelium give rise to most of

the senile degenerations of the macular choroid and retina which cause loss of central vision in the aged.

The most commonly seen senile change in the choroid is the deposition of hyaline masses in the lamina vitrea of the choroid. These yellowish-white spots have been variously designated as "drusen," colloid bodies, hyaline bodies, and guttate choroiditis or guttate degeneration. When they are small and scattered they cause no disturbance of vision and are considered to be of no significance. When they become larger, and especially when they are massed in the macular region, however, they may cause pressure atrophy of the overlying rods and cones with resultant serious impairment of vision. According to Duke-Elder,<sup>7</sup> such cases were first described by Hutchinson<sup>11</sup> under the name of "symmetrical central choroidoretinal disease occurring in senile persons and later came to be known as Tay's central guttate choroiditis." Recently attention has been called again to the significance of these lesions by Gifford and Cushman,<sup>9</sup> who include Doyne's honeycomb choroiditis among lesions of this type. They prefer the term "central retinopathy due to hyaline deposits of the lamina vitrea." They believe that at least some of the cases of central disk-shaped retinopathy or disciform degeneration of the macula (Kuhnt-Junius)<sup>16</sup> develop on the basis of hyaline degeneration of the lamina vitrea. These cases have usually been ascribed to arteriosclerosis of the retinal or choroidal vessels. However, according to Gifford and Cushman,<sup>9</sup> Behr<sup>3</sup> "found degenerative changes in the lamina vitrea with a number of vessels growing through it into the choroid. He expressed the belief that the primary cause of the condition was a senile or nutritional defect in the elastic system of the choriocapillaris and lamina vitrea which allowed a transudate to collect beneath the pigment epithelium. From this the connective tissue developed by proliferation of pigment epithelium and growth of new vessels from the choroid." As a result of their investigations of a case presenting a juvenile form of central disciform retinopathy, Gifford and Cushman<sup>9</sup> obtained evidence in support of the location of the primary lesion in the lamina vitrea rather than in the vascular system. They stated that "evidently complete defects in the membrane may not be necessary for the production of the typical condition but only an altered permeability which permits fluid from the choriocapillaris to collect beneath the pigment epithelium. Proliferation of the pigment epithelium seems to result from such a transudation. If vessels from the choroid do not grow into the resulting mass, hemorrhages may be absent from the picture." In this conception of the origin of the lesion, it is probably necessary for the occurrence of hemorrhage, that complete defects exist in the lamina vitrea to allow the growth of vessels from the choriocapillaris into the transudate under the pigment epithelium.

In their cases, Gifford and Cushman<sup>9</sup> did not find more marked evidences of hypertension and generalized arteriosclerosis than the average in the age group affected. Nor were there any marked evidences of retinal arteriolosclerosis. They favor the conception of a senile or pre-senile degeneration of the lamina vitrea belonging to the so-called abiotrophies of Treacher Collins. Yudkin<sup>21</sup> suggested that deficiencies or disturbances of nutrition in early life might be a factor in the subsequent development of drusen, but Gifford was inclined to disagree with this suggestion. That some factor other than age alone may be involved in the production of these hyaline degenerations is suggested, of course, by their occurrence not infrequently in young people in whom they "may be due to some obscure general or local metabolic disturbance" (Duke-Elder).<sup>7</sup>

In his discussion of Gifford and Cushman's paper, Verhoeff<sup>20</sup> stated his belief that the cause of disciform degeneration of the macula is hemorrhage beneath the pigment epithelium of the retina in the senile form and serous exudation in the same location in the juvenile form. He thinks that senile degeneration of the pigment epithelium is the primary change which predisposes to hemorrhage from the chorioecapillaris and that the lamina vitrea is only slightly affected. In the end, this difference of opinion may be only a matter of the extent of visible damage to the lamina vitrea in the sections examined since hyaline changes in the lamina vitrea are supposed to be the product of primary degenerative changes in the pigment epithelium. The senile type of colloid spots, or drusen, it is generally agreed, are due to the deposition of hyaline material in the euticular layer of the lamina vitrea as a result of metabolic disturbances in the pigment epithelium with excessive secretion of the hyaline substance (Rones).<sup>16</sup> Rones thought that the "degenerative" type of drusen was formed by the actual transformation of degenerated pigment epithelium cells. However, Verhoeff<sup>19</sup> was inclined to think that all forms of drusen were due to the deposition of hyaline material beneath the pigment epithelium.

Another lesion of the macular retina in old people which is presumed to depend on primary damage to the choroidal circulation is the senile macular degeneration of Haab which is stated by Duke-Elder<sup>7</sup> to be "characterized by the presence of degenerative changes, usually punctate in nature, occurring bilaterally, limited to the region of the macula, and due to sclerosis and obliteration of the chorioecapillaris in the central area." The ophthalmoscopic findings are limited in the early stages to a mild pigment stippling of the macular retina which appears to be insufficient in amount to account for the loss of vision. Later there is evident decoloration of the fovea due to the sclerosis and atrophy of the underlying chorioecapillaris and still later hemorrhages and transudation may occur in the retina and choroid so that the ultimate picture may be essentially indistinguishable from that seen in other forms of choroidal sclerosis and atrophy or even in disciform degeneration of the macula. Correspondingly, the histologic changes in the advanced stages comprise sclerosis and hyaline degeneration in the vessels of the choroid, especially of the chorioecapillaris, colloid exudates of the lamina vitrea, exudation under the pigment epithelium, atrophy and proliferation of the pigment epithelium, and atrophy and disorganization of the deeper layers of the retina with essential destruction of the rods and cones.

Retinitis circinata (circinate retinopathy, or circinate degeneration of the retina) is another lesion which occurs usually in elderly individuals and which is often ascribed to changes in the choroidal circulation. According to Duke-Elder,<sup>7</sup> it was described originally by Hutchinson<sup>11</sup> under the title "symmetrical central choroido-retinal disease occurring in senile persons" and is characterized by "a girdle of bright white spots in the deeper retinal layers around the central area and the development of degenerative changes at the macula." The characteristic white exudation observed ophthalmoscopically is represented histologically by sero-albuminous, hyaline or fatty degenerative changes in the internuclear and internal nuclear layers of the retina. These were considered originally by Amman to be the residuals of hemorrhagic extravasation. But it seems more probable, as noted by Morax<sup>13</sup> and Seefelder,<sup>18</sup> that the exudation is essentially similar in nature to that seen in hypertensive retinopathy and is dependent primarily on impairment of the circulation as the result of arteriosclerosis either in the retina or, as suggested by Seefelder,<sup>18</sup> in the

choroid. In Seefelder's case, there was an associated advanced cystoid degeneration of the macular retina with complete disappearance of the outer layer and the layer of rods and cones. Senile macular degeneration was present also in the other eye of Seefelder's patient.

According to Berens,<sup>4</sup> 4 clinical types of central senile chorioretinitis were described by de Schweinitz.<sup>17</sup> In Type I "the macula may appear darker than normal, it may be delicately mottled or white glistening spots may be seen." In Type II "yellowish white spots in the macula are interspersed with pigment dots and occasionally with hemorrhages." In Type III "a round or slightly oval hole appears in the macula." In Type IV "grayish or greenish heaped exudates, which may resemble tumors and hemorrhages, may be present." It seems probable that Types I, II and IV represent various phases in the progressive development of senile macular degeneration (Haab)<sup>9a</sup> as described previously. Probably Type III belongs more properly in the group of cystoid degenerations of the retina.

It is well known that the senile retina has less luster and gives off fewer light reflexes than that of the child and young adult. Duke-Elder<sup>7</sup> states: "In the aged the retina shows less marked evidences of senility than most tissues. As a whole it becomes less transparent owing largely to an increase in the neuroglial elements, a process which is associated with a parallel tendency to atrophy of the neural elements." In some instances, "the whole thickness of the retina may undergo fibrous transformation and cystic degeneration." This occurs usually in cases in which there is sclerosis and atrophy of the choriocapillaris. Quoting again from Duke-Elder,<sup>7</sup> "Cystic or cystoid degeneration of the retina is properly a passive degenerative process wherein gaps are formed within the tissue owing to the disintegration of its neural elements." It is difficult to distinguish clinically, however, between the cysts which have developed as a primary degenerative process and those which are the end-result of vascular, edematous, traumatic and inflammatory lesions. Senile degeneration is one of the more common causes of the development of cystic spaces in the periphery of the retina and is a somewhat less frequent source of the formation of macular cysts and holes, according to Rones.<sup>16</sup> In the early stages, small spaces appear in the outer nuclear layer. Similar spaces then become visible in the inner nuclear layer, and for a time these two layers are separated by a thin septum. The disappearance of this septum causes the retina to appear as two thin sheets with delicate membranes stretching between them, forming large cystic spaces. On section, these spaces are usually found to be empty, though at times an albuminous coagulum is seen in them. The membranes stretching between the two limiting membranes of the retina are the stretched elongated Mueller's fibers, the true retinal layers having atrophied. Holes in the retina are formed by the breaking down of the limiting membranes. Duke-Elder<sup>7</sup> states that "cystic degeneration occurs with great regularity to a well-marked degree in the normal eyes of old people. It is the rule also in myopic degeneration (Hannssen, 1925)<sup>9b</sup> in which condition it is not uncommon even in comparatively early life (Ochi, 1927).<sup>13b</sup> Iwanoff (1865)<sup>11a</sup> found no instance in 20 globes of children under 8, in 12% of patients between 20 and 40, and in 50% of eyes between 50 and 80 years."

Cystoid degeneration of the peripheral part of the retina can rarely be recognized ophthalmoscopically and its only clinical importance is its possible rôle in the production of detachment of the retina. Cystoid degeneration of the macular retina, however, is of considerable clinical

importance since it eventuates always in loss of central vision. Duke-Elder<sup>7</sup> states: "At first, cystic spaces are formed in the retinal substance which may be visible, particularly with red-free light with which a typical honeycomb appearance is produced of small flecks in the central area (the vesicular macular edema of Nuel, 1908); eventually on rupturing these may produce a depression in the central area if they are confined to the inner layers of the retina, or a complete macular hole if the whole thickness of this tissue is involved (the retinitis atrophicans of Kuhnt, 1900). A hole in the macular retina is recognized ophthalmoscopically as a dark red circular punched out appearing spot about  $\frac{1}{4}$  to  $\frac{1}{3}$  disk diameter in size, usually directly in the fovea but occasionally slightly eccentrically placed. Its edges are usually clean cut but occasionally slightly ragged residuals of the anterior wall of the cyst are seen. At times the lesion is recognized in the stage of a thin walled cyst before rupture occurs. The dark red color of the lesion is due to increased visibility of the underlying choroid. Around a recently developed hole a halo of mildly edematous retina may be seen at times. Flat or shallow detachment of the retina surrounding the hole may develop in some instances. Probably the majority of macular holes are of traumatic origin, resulting from contrecoup rupture or from concussion or contusion edema of the retina. However, a fair number of cases are seen in elderly individuals, in whom they may develop as a sequence of simple senile cystoid degeneration of the retina or of the atrophy of the retina secondary to sclerosis and atrophy of the choriocapillaris, and occasionally as a complication of chronic edema of the retina resulting from circulatory insufficiency."

According to Rones,<sup>15,16</sup> the pigment epithelium of the retina is affected early by senile changes in the choroidal vessels and the resultant atrophy of the choriocapillaris. There is both degeneration and proliferation of the pigment cells and the pigment granules become small and round in form and are scattered over the basal membrane causing an irregularity and clumping of the pigment ophthalmoscopically. Associated with this there may be "an atrophy of the retinal periphery, with a decrease in the nerve fibres, ganglion cells, and inner nuclear layer, together with a disappearance of the rod and cone elements and a hypertrophy of connective tissue. The pigment epithelium becomes sparser, and pigment granules wander into the retina." This pathologic process undoubtedly explains the pigment migration into the periphery of the retina visible ophthalmoscopically in many elderly individuals, especially in those with cardiovascular disease, which is often sufficient in amount to simulate familial pigmentary degeneration of the retina or healed choroidoretinitis. In some instances, these changes may be associated with concentric contraction of the fields of vision. In most cases, however, there is no subjective disturbance of vision.

The subject of arteriolosclerosis and arteriosclerosis involving the vessels of the retina cannot be considered in detail here. Its proper consideration would require a separate and individual review. In very brief fashion, the view of Rones<sup>15,16</sup> may be quoted: "When the arterioles of the body (including those in the retina) are involved in the diffuse type of hyalinization of the media, there are found as ocular complications a diversity of hemorrhages and exudates, while general bodily resultants are hypertension and cardiac and renal impairment. . . . Pathologically the changes (of arteriosclerosis) occur in the intima of the larger retinal vessels. The intima shows an increase in thickness due to a proliferation of connective

and elastic tissue, with a deposition of lipoids. This thickening is usually of a nodular character, so that small plaques project into the lumen of the vessel causing localized constrictions of its calibre. Clinically, this pure type of arteriosclerosis is observed in elderly individuals without any associated hypertension." This type of arteriosclerosis seems to be rare except in the central artery of the retina in the optic nerve or in its primary branches on the papilla where it may be the cause of some cases of obstruction of the central artery.

In the words of Rones,<sup>15,16</sup> "The eye is subject to changes in all of its tissues with advancing age. . . . It is important to be able to recognize these senile variations clinically, so as to distinguish them from pathological lesions (*sic*). However, this is often a difficult matter, for numerous diseases of the eye are most common in those years when senile changes occur, and it is of interest to attempt to relate the aging phenomena to the etiology of the disease. . . . It must be borne in mind that the relationship between tissue age and chronological age is not an exact one. Some individuals have advanced senile changes in their tissues at 40 years of age, while in others there is singularly little evidence of this at 80. . . . Nevertheless it will be found that in every individual past the age of 40 years the ocular tissues will show alterations that fall into the senile classification, even though they are only in the beginning stages." In the causation of all these degenerations, the changes in the vascular system seem to be of primary and fundamental importance.

Many authorities are of the opinion expressed by Behr<sup>3</sup> that the macular degenerations, which are the most serious of the so-called senile lesions of the retina and choroid from the standpoint of visual disability, are essentially abiotrophic, that is to say a more or less inherited or familial wearing out of the tissue at a certain but variable age. However, Berens<sup>4</sup> calls attention to the important rôle played by changes in circulation and in the vascular system, some of which may be prevented, controlled, or at least moderated by the prophylactic or early removal of infections, focal or otherwise, and the avoidance of overindulgence in alcohol or tobacco, especially in advancing years. In Heath's<sup>10</sup> opinion a low fat and carbohydrate diet is of importance in the control of certain macular degenerations. Piersol<sup>14</sup> notes that evidence is being accumulated to show that diet plays an important part in human longevity and that a well-balanced, optimal diet, rich in vitamins during youth and early adult life, is important in preventing or delaying the onset of senile tissue changes. In addition to the advisability of proper dietary habits, Berens<sup>4</sup> emphasizes the importance of hygienic living, adequate rest and sleep, sunshine and moderate exercise adjusted to the individual's capabilities. In conclusion, the words of Piersol<sup>14</sup> are well worth quotation: "Heretofore, the physician has somewhat lacked interest in disease as it appears in the older person, looking on it as irremediable and inevitable. If the medical problems of old age are to be successfully met, he must abandon his defeatist attitude and approach the management of such problems in a spirit of greater constructiveness and optimism. It will then be possible for him to do much to prolong life, relieve suffering and mitigate in no small degree the handicaps and infirmities of old age. . . . If the physician is to meet his obligations to the aged, he must constantly bear in mind the importance of controlling those presenile states which if allowed to go unheeded will lead to chronic visceral disease, for which little can be done."



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## PHYSIOLOGY

## PROCEEDINGS OF

## THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF DECEMBER 21, 1943

**Roentgen ray Diffraction Patterns of Striated Muscles (Effects of Stimulation, Rigor, Drugs).** M. SPIEGEL-ADOLF, E. W. ASHKENAZ, KATHRYN McHALE, Fellow, A. A. U. W., and G. C. HENNY (Departments of Colloid Chemistry and Physics, Temple Medical School). Former Roentgen ray diffraction studies of globular proteins were extended to studies of parallel fibers of sartorius muscles of *Rana pipiens*. Our apparatus permits the study of living material under various conditions, the exposure time being cut down to 6 minutes. While powdered dried muscle shows only 2 diffuse rings, identical in their spacings to the usual protein pattern, wet and dried muscle fibers indicate orientation in both rings, perpendicular to the long axis of the fibers. A comparison between the patterns of wet and dried muscles based on statistical computations indicates that only a small amount of water is located intramolecularly. The reversibility of the changes produced by drying is practically complete. Stretching of the muscles by weights below the breaking point produces an additional well-defined diffraction line, identical with one observed in stretched frog tendon and also with a similar line in powdered muscle nuclei.

Electrical stimulation, caffeine poisoning and mechanical stimulation cause disappearance of orientation in the Roentgen ray diffraction pattern if the muscle is allowed to shorten. Conversely, after electrical stimulation, stretching of such a contracted muscle restores the orientation. Rigor-inducing procedures (death, heat, chloroform) show definite changes of the Roentgen ray diffraction patterns: disappearance of orientation in all instances, characteristic sharpening of back-bone reflection, new spacing

upon heating and increased membrane permeability, as in chloroform poisoning. Lactic acid produces similar losses in orientation in concentrations over 0.005 N; hypertonic salt solutions produce a loss in orientation in immersed, cut muscles, parallel to the known loss in birefringence. Such muscles, after washing and drying, show salt rings superimposed upon their own diffraction patterns, thus indicating an increased permeability of the cell membranes. Similar changes can be observed in muscle in which the NaCl of Ringer's solution has been replaced by KCl, showing thus its biologic effectiveness.

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**Insect Nerve Sheaths and Insecticide Penetration.** A. GLENN RICHARDS, JR. (Zoölogical Laboratory, University of Pennsylvania). Insect nerves are non-medullated but are surrounded by a bound lipid sheath of submicroscopic thickness. The lipids are quickly released and extracted by alcohol but the fiber tract areas can be stained with Sudan after formalin fixation. Polarized light studies on living nerves show the presence of metatropic sheaths, the entire nerves having the closest balance of positive and negative components of any known nerves. Insect nerve cords exhibit a high degree of photoelasticity, and the increase in amplitude of birefringence is a fairly constant reading. Chemicals which cause a decay of the axoplasmic birefringence also decrease or destroy the elasticity.

The distribution of the lipid sheaths is positively correlated with the penetration of oils and oil-solvents from trachæ as shown by selective staining from lipid dyes used to mark the material applied. It seems likely therefore that these lipid sheaths condition the penetration of such materials into the nervous system, at least when the materials are diffusing from trachæ.

Extensive studies now being performed show that the various neurotoxic insecticides may have different effects on the optical and elastic properties. Lipid solvents eliminate the lipid sheaths; some insecticides cause a particulate degeneration of the lipids, others attack the axoplasm, and others may affect both axoplasm and sheath in different degrees. For insect nerves where the proteins and lipids are so closely balanced optical analyses are far more sensitive than ordinary histopathologic methods.

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**Studies on Bioassay of Penicillin.** LIDA F. HOLMES and JOHN S. LOCKWOOD (Harrison Department of Surgical Research, University of Pennsylvania). Foster and Woodruff (*J. Bact.*, 46, 187, 1943) have described a method for assay of penicillin, testing turbidity of a staphylococcus culture. A method based on the same principle has been devised in this laboratory which requires only a 2 hour period of incubation and which will detect concentrations as low as 0.01 unit per cc. The test can be adapted to examination of colored fluids containing penicillin by centrifuging down the staphylococci and resuspending them in distilled water for reading their turbidity.

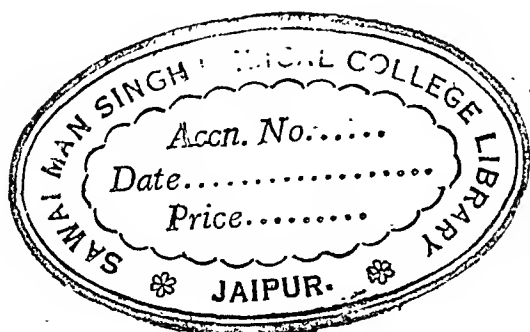
The object of our investigation was to find a sensitive, rapid test for determination of penicillin levels in serum. However, when we conducted turbidimetric tests with serum containing known added amounts of penicillin, several difficulties were encountered: (1) Natural antibody to the staphylococcus causes inhibition of growth in certain dilutions of serum.

(2) It was found that serum interfered with the action of penicillin. Likewise, horse and rabbit serum and human ascitic fluid contain an anti-penicillin factor. Dialysates of these fluids are also antagonistic to penicillin, and the inhibiting substance may be concentrated by lyophilization of the dialysates. This unknown anti-penicillin substance resists heating at 60° C. for 30 minutes, but is destroyed by boiling for 10 minutes. Investigation of its properties is being continued.

**The Use of Horse Brain Thromboplastin for the Quantitative Determination of Prothrombin.** LOUIS A. KAZAL and L. EARLE ARNOW (Department of Biochemistry, Medical Research Division, Sharp & Dohme, Inc., Glenolden, Pa.). Preparations of horse brain thromboplastin, prepared essentially according to Quick's directions for rabbit brain, clotted citrated human plasma in 16.8 to 25.8 seconds, when optimal concentrations of calcium chloride solution and thromboplastin were used in the Quick test. The optimal concentration of calcium chloride solution was 0.184% for the plasma used by us. Dilutions of the brain suspension with an equal volume of water yielded preparations that gave minimal clotting times.

Although rabbit brain thromboplastin is more active than horse brain thromboplastin, it appears that the horse brain preparation is suitable for the quantitative determination of prothrombin. With plasmas of known prothrombin contents, determinations usually could be made to within 9% of the theoretical concentration of prothrombin. The differences in prothrombin concentrations found with the two thromboplastins were not greater than 6%. Except in 1 case, with plasmas of unknown prothrombin concentration the difference in the concentrations determined with horse and rabbit thromboplastins was not greater than 13%.

Horse brain thromboplastin has been lyophilized and then stored under vacuum at 5° C. for 14 months with little or no loss of potency.



# BOOK REVIEWS AND NOTICES

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THE COMMON FORM OF NIACIN AMIDE DEFICIENCY DISEASE: ANIACINAMIDOSIS. By WILLIAM KAUFMAN, PH.D., M.D. Pp. 62. Bridgeport, Conn.: Yale University Press, 1943. Price, \$3.00.

UNDER the name of a specific vitamin deficiency called aniacinamidosis—probably because niacinamide is the substance used for its therapy—is described a complex of almost innumerable symptoms ranging from neurasthenic manifestations to calluses and corns. Nothing that could be called valid scientific evidence is to be found in the entire 58 pages, but it is written in a soothing, pseudoscientific manner that might easily deceive the uninformed.

E. W.

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FINGER PRINTS, PALMS AND SOLES. An Introduction to Dermatoglyphics. By HAROLD CUMMINS, PH.D., Professor of Microscopic Anatomy, Tulane University, School of Medicine; and CHARLES MIDLO, M.D., Associate Professor of Microscopic Anatomy, Tulane University (Formerly Assistant Professor of Anatomy, Louisiana State University). Pp. 309; 149 illus. Philadelphia: The Blakiston Company, 1943. Price, \$4.00.

THE chief purpose of the authors in writing this book was "to fill the want of a comprehensive treatise on dermatoglyphics." They have presented the subject under the following divisions: Part 1, Orientation; Part 2, Methodology and Description; and Part 3, Biology. Part 1 might have been improved and its seriousness more appreciated if the brief remarks on current popular interest in finger printing and the dactylomaney incident had been omitted. Part 2 contains the methods and procedures necessary for anyone actively working in the field. Much of this part is covered adequately in books already existing but its inclusion in this book is useful in interpreting portions of Part 3. It is in the last part where the scientific importance of the work lies. Here in about one-half the book and in an interesting and effective manner the authors have marshaled the available data on the fundamental biologic phases of dermatoglyphics. This publication should find wide usefulness among students of biology and workers in identification.

R. W.

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A TEXTBOOK OF MEDICINE. Edited by RUSSELL L. CECIL, A.B., M.D., Sc.D., Professor of Clinical Medicine, Cornell University Medical College, etc., Associate Editor for Diseases of the Nervous System; and FOSTER KENNEDY, M.D., F.R.S.E., Professor of Clinical Neurology, Cornell University Medical College, etc. Sixth ed., revised and entirely reset. Pp. 1566; 195 illus. Philadelphia and London: W. B. Saunders Company, 1943. Price, \$9.50.

THIS already excellent text has been still further improved by changes in the format (such as the 2-column, slightly larger page), and in the text. The Preface lists 11 new articles on subjects not covered in previous editions, 31 new treatises on subjects previously covered, and 2 new features—introductory chapters to groups of diseases (*e. g.*, Rickettsiae, Pneumococcic Infections, Metabolism, the Blood, etc.); and a list of normal values for the commoner, but constantly growing laboratory tests. All this is a direct reflection of the efficient industry of the Editor, without which a single volume composed by so many authors would be almost surely a failure, even with the eminent list of authors that this work has gathered together.

E. K.

**PHYSIOLOGY OF THE NERVOUS SYSTEM.** By JOHN F. FULTON, M.A., D.Phil., D.Sc. (Oxon.), S.B., M.D., Sterling Professor of Physiology, Yale University; formerly Fellow of Magdalen College, Oxford. Second ed. Pp. 614; 112 figs. New York: Oxford University Press, 1943. Price, \$9.00.

THIS edition of Fulton's book should enjoy great popularity with the medical student (because of the clarity with which complicated mechanisms are presented), with the physician (because of the stress laid upon the practical applications of the material presented), and with the physiologist (because of the completeness of the text and its 66 page bibliography). It has been brought up to date by Dr. Fulton and his collaborators, particularly in the chapters dealing with the neurohumoral theory of nerve excitation and with respiration. A more detailed discussion of the effects of oxygen lack and cerebral anemia upon the nervous system as a whole would have been useful in wartime, with its attendant high altitude flying, dive bombing, and exsanguinating wounds.

J. C.

**MEDICAL CLINICS ON BONE DISEASES.** A Text and Atlas. By I. SNAPPER, M.D., formerly Professor of Medicine, Peiping Union Medical College, Peiping, China. Pp. 225; 30 plates. New York: Interscience Publishers, Inc., 1943. Price, \$10.75.

DR. SNAPPER's book is a very interesting résumé of bone diseases, taking up in great detail von Recklinghausen's disease, Hodgkin's disease of bone, multiple myeloma, lipoid granulomatosis, osteomalacia, and other diseases involving the skeletal system. The author has had an exceptional opportunity in his wide clinical experience as former Professor of Medicine at the Peiping Union Medical College in China. Due to his familiarity with the frequency of these bone diseases, both in America and China, he is able to discuss very interestingly the pathogenesis and the relation of many of these diseases regarding their disturbed metabolism, their biochemical problems and the diseases of deficiency. The Reviewer enjoyed reading the text of this unusual compilation of rather rare bone diseases. The very beautiful plates with which his book is illustrated are especially deserving of mention. The clearness of detail reproduced both from the Roentgen rays and photomicrographs of the cases under discussion are an indication of the huge amount of clinical material that must have been at his disposal.

P. C.

**THE CONQUEST OF EPIDEMIC DISEASE.** A Chapter in the History of Ideas. By CHARLES EDWARD AMORY WINSLOW. Pp. 411. Princeton, N. J.: Princeton University Press, 1943. Price, \$4.50.

THIS story of man's epic struggle against epidemic disease is more than a fascinating saga—it is a study in human evolution. So dramatic and recent has been the conquest of the grim horseman of pestilence that the gradual growth of concepts through plague-ridden centuries of dreadful experience is prone to be forgotten. Public health victories during the last hundred years are familiar themes, but Professor Winslow has reviewed the records of 3000 years and under his skilful pen the slow emergence of social consciousness of the controllable nature of contagious disease takes form—not by propounding but compounding the slow-won facts to the strategy of community effort rather than individual medical care which has brought plagues under control.

The reconstruction of mental attitudes toward the external cause of contagious disease—from primitive notions of malignant demons, the wrath of God, astrologic influences, naturalistic concepts of Hippocrates, epidemic constitution of schoolmen and primitive biologic experiments, to the germ theory of disease, which gave instruments for sanitary adjustment of our environment through processes of civilization—is a study in mental evolution. Unconsciously, perhaps, one recognizes the headlands of progress, not so much in

the scholarly deductions of authorities as in the simple experiments of novices. Even the astute comprehension of Fracastorius as to the essential nature of contagion could not be assimilated by the intellectual and spiritual leaders of the time, and 300 years of evolution were required for the great sanitary awakening in the first half of the 19th century, and for the conception of *contagium animatum* of Kircher, Redi and Leeuwenhoek to generate a biologic theory of disease. The discoveries unleashed by the germ theory constitute a glorious chapter in science, and the technical application of these discoveries during the present century is one of the great achievements in human welfare.

The book is thus of general interest and a *must* for every student of public health and welfare and a *should* for every intelligent person. It is a tale told to quicken the dulllest imagination and should adorn the shelves of the most modest library.

W. W.

**MICROSCOPIC TECHNIQUE IN BIOLOGY AND MEDICINE.** By E. V. COWDRY, Professor of Anatomy, Washington University, and Director of Research, The Barnard Free Skin and Cancer Hospital. Pp. 206. Baltimore: The Williams & Wilkins Company, 1943. Price, \$4.00.

THE author presents a new type of book for investigators in the field of biology and medicine who are interested in techniques for the preparation and micro-examination of tissues, animal parasites, protozoa, bacteria, rickettsiae and viral elementary bodies.

The text is arranged in alphabetical order with headings containing cross-references to other titles appearing in the text. Each heading is discussed, not in voluminous detail, but in concise manner giving exact and specific information. It is not intended to be an encyclopedia but a ready reference text on recognized physical, chemical and biologic means for studying the minute structure of living matter.

Great emphasis is placed on fixatives, dyes and stains, microdetection of enzymes, methods of pH determination, histo-pectroscopy, microincineration techniques, microchemical reactions and *in vivo* examination of tissue. Especially important are the references to the literature where more complete articles and reviews of the individual subjects may be found as well, as references to good texts on each subject.

F. E.

**DOCTOR IN THE MAKING. The Art of Being a Medical Student.** By ARTHUR W. HAM, M.B., Associate Professor of Anatomy, in Charge of Histology, Faculty of Medicine, University of Toronto; Honorary Secretary of the Banting Research Foundation; and M. D. SALTER, M.A., Ph.D., Lecturer and Research Fellow in the Department of Psychology, Faculty of Medicine, University of Toronto. Illustrations by JEAN McCONNELL. Pp. 179. Philadelphia, London and Montreal: J. B. Lippincott Company, 1943. Price, \$2.00.

Two Canadian medical teachers, an anatomist and a psychologist, have combined to put before medical students and those considering a medical career the motives and medical equipment that promote progress and the common enemies that hinder success. We wish that this review would fall into the hands of these two groups; the time required to read and really possess its contents could not be better spent otherwise. Study habits, attitudes toward medical topics and toward those doing the instructing, general personality characteristics and ways of improving them, understanding and applying the scientific method, the proper allocation of time—such are the topics simply and clearly set down for consideration.

Especially appealing to the Reviewer is the chapter on the importance of pre-medical and pre-clinical subjects—"There is no mysterious course in later years of medical school that teaches you to become a doctor." Training for practice begins before entering school, and continues throughout life; but it is

especially in the earlier courses—and only there for the vast majority—that the basic knowledge and attitudes can be obtained on which the new edifices of knowledge can be erected that are required to keep a physician “up to date.”

How not to study or waste the time spent in evening work, how to assimilate lectures, how to organize one's knowledge, how to understand (as opposed to memorizing), and to appreciate the meaning of words, these are all matters which, to the Reviewer after a considerable experience in teaching medical students, are of prime importance in the student's self-education, not to mention the passing of examinations. How to use textbooks and journals and the medical library, the importance of terms and definitions (*i. e.*, semantics) are phases that are particularly lacking in the equipment of most students.

The chapter, *Your Child-Self*, really a bird's-eye view of the practical psychology of the individual, could be studied with profit by medicos of every age and condition.

In short, this is an excellent aid on “the right road to learning.” Incidentally, we hope that its wartime paper will stand the strain of heavy usage. One could wish that Lippincott's had an endowment fund that would place a copy in the hands of every pre-medical student, if it were not that things so acquired are apt to be correspondingly evaluated. Last but not least, the student should be attracted by its brevity.

E. K.

**URINE and URINALYSIS.** By LOUIS GERSHENFELD, P.D., Ph.M., D.Sc., Professor of Bacteriology and Hygiene and Director of the Bacteriological and Clinical Chemistry Laboratories at the Philadelphia College of Pharmacy and Science. Second ed. Pp. 304; 42 figs. Philadelphia: Lea & Febiger, 1943. Price, \$3.25.

In this new edition there is a chapter on the history of methods of analysis of urine which forms an excellent background to the work that the author wishes to present. The physiology of the urinary tract is discussed to promote better correlation of the laboratory tests to be described. The most modern methods of qualitative and quantitative analysis of urine are presented in detail. Normal and abnormal findings of these examinations are discussed.

In addition to the chemical examinations themselves, there are chapters on the bacteriologic and microscopic analysis of urine, as well as renal function tests. The chapter on analysis of urinary calculi presents a method which has been generally acceptable, but is actually obsolete in the light of recent publications on the subject.

An appendix gives a description of the apparatus and reagents used in urinalysis.

Here arranged in a single volume is information concerning the urine which could otherwise be obtained only by covering many texts and papers on the subject. There is nothing new or astounding about the information presented; however, it seems justifiable to have such a volume for the use of the busy pharmacist, chemist, bacteriologist, technician or physician.

L. La T.

**REACTION TO INJURY.** Pathology for Students of Disease Based on the Functional and Morphological Responses of Tissues to Injurious Agents. By WILEY D. FORBUS, M.D., Professor of Pathology, Duke University and Pathologist to the Duke Hospital. Pp. 797; 523 illus. (20 in color). Baltimore: The Williams & Wilkins Company, 1943. Price, \$9.00.

THIS fine book is an important contribution to pathologic literature. The difficult subject of inflammatory diseases is beautifully organized. A new, but most logical, approach to this problem is used. Pathogenesis is emphasized, etiology being delegated to a secondary place. This should be of distinct advantage to the student, to whom the variety of inflammatory responses often seems infinite.

The book is planned around this basic definition: the essential element in disease is the reaction of the cells of the body. This reaction may be of 3 types: (1) by active resistance; (2) by passive submission; (3) by effecting an adaptation. In Part I these are discussed and all types of injurious agents are considered—trauma, chemical and physical agents, bacteria, fungi, protozoa, worms and so forth. Part II, the body of the book, a discussion of the active resistance type of reaction, includes detailed descriptions of bacterial infections, rheumatic fever, nephritis, virus diseases, and granulomata, to take a few examples. Unfortunately, Parts III and IV, a discussion of the submissive and adaptive reactions, are not completed as yet. A second volume is projected for these.

The book is magnificently printed and illustrated. Nearly 90% of its 532 figures are original, many in excellent color.

Many pathologists may object to the strong teleologic flavor of some of the terms used. These are often difficult to eliminate without sacrificing emphasis and clarity.

As a special text on inflammatory diseases, this book should prove extremely valuable to the student of pathology. As the work limits itself to this special field, the 2d-year student must naturally use it in conjunction with a standard text. With this book, however, Dr. Forbus has made a fresh and valuable contribution to the pathologic literature of inflammatory disease. W. S.

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ADDENDUM TO THE CHEMISTRY OF THE AMINO ACIDS AND PROTEINS. Edited by CARL L. A. SCHMIDT, M.S., PH.D., Professor of Biochemistry and Dean of the College of Pharmacy, University of California. This is a supplement to the Second edition. Pp. 155; figures and tables. Springfield, Ill., and Baltimore, Md.: Charles C Thomas, 1943. Price, \$5.00.

As the title implies, this book is not a revision of the original volume of *The Chemistry of the Amino Acids and Proteins*. Instead it is more in the nature of an addition, even to the extent of continuing the reference and page numbering from the original. The chapter divisions and order are unchanged except in a few cases in which there was no revision. For the most part the authors are also the same. The chapter on nutrition has been revised by Dr. H. J. Almquist, instead of Dr. R. W. Jackson, and Dr. C. L. A. Schmidt has taken over the work of several of the former authors especially those from foreign countries. As in the original volume, the various chapters are written in a highly technical manner and in many cases data from original articles have been included. The book is of particular value to those working in and familiar with the field of protein chemistry, but those whose major interests lie in other branches of biochemistry will also find this book of value. J. J.

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PRINCIPLES AND PRACTICE OF REHABILITATION. By JOHN EISELE DAVIS, M.A., Sc.D., Veterans Administration Facility, Perry Point, Md. Pp. 211; various tables and charts. New York: A. S. Barnes & Co., Inc., 1943. Price, \$3.00.

This experienced rehabilitator tells us that the modern approach to our problem is medical, psychologic, psychiatric and vocational. Among other chapters are those on Effect of War and Depression; Psychiatric Approach, General Consideration; Elemental Principles of Mental, Nervous and Physical Reconstruction; Therapeutic Objectives and Results; Handicraft and Art. The recreational discussions are among the best given, particularly those of Interest and Effort Theories, wherein are reports on dementia præcox, manic depressive and dementia paralytica patients. A useful feature is an Appendix giving 6 case histories from the New Jersey Rehabilitation Clinic. No mention is made of the Rorschach test, which has both diagnostic and prognostic value.

N. Y.



**PYE'S SURGICAL HANDICRAFT.** Edited by HAMILTON BAILEY, F.R.C.S. (ENG.), Surgeon, Royal Northern Hospital, London; Surgeon and Urologist, Essex County Council; Surgeon, Italian Hospital; Consulting Surgeon, Clacton Hospital and the County Hospital, Chatham; External Examiner in Surgery, University of Bristol. Thirteenth ed. Pp. 536; 534 illus. Baltimore: The Williams & Wilkins Company, 1942. Price, \$6.00.

THIS is really a manual of minor surgical techniques. The scope extends from laboratory technique, bandaging, and first-aid through anesthesia and general surgery to minor surgical procedures of otolaryngology, ophthalmology, and dentistry and to the treatment of gonorrhea and syphilis. The purpose of the book is commendable in that it presents many of the techniques that are not included in ordinary textbooks and are learned by the intern only through observing the methods of older members of the profession.

The scope of material is apparently so extensive that it prohibits an adequate discussion of many of the individual subjects. The sections on fractures, although very brief, are excellent.

H. Z.

**A MEDICAL BIBLIOGRAPHY.** A Check-list of Texts Illustrating the History of the Medical Sciences. Originally compiled by the late FIELDING H. GARRISON, M.D., and now revised, with additions and annotations by LESLIE T. MORTON, Librarian, St. Thomas's Hospital Medical School. Pp. 412. London: Grafton & Co., 1943. Price, £2.10s net. (About \$10.00).

THIS useful book has an interesting provenance. The underlying idea goes back, like so many others, to William Osler, who suggested the compilation of such a list to Garrison. This appeared in the Surgeon-General's Index Catalogue in 1912 (vol. 17, Sec. S, pp. 89-178) and served its compiler "as a convenient scaffolding" for his great *History of Medicine*, which first appeared in 1913. A revised and enlarged list appeared in the Bulletin of the Institute of the History of Medicine in 1933, to which 1680 new entries have now been added by the English compiler. Thus Canadian, American and English scholars have collaborated to our advantage. This edition also contains many useful annotations, and—especially valuable in a work of this sort—good author and subject indices. It should be noted that the references are to entry numbers and that only the references to original works are given in Roman type.

The value of such a volume on one's shelves is considerably enhanced to the user if he early takes care to develop a sufficient acquaintance with its plan of construction to be able easily to identify the item sought. Thus, the simple fact that in each section the works are arranged in the chronologic order of their appearance at once changes an apparent heterogeneous assemblage into a simple and useful orderly list. The list of over 50 "Principal Headings" (p. vii) ranges from voluminous subjects such as Anatomy and Physiology (1072 references) to Epidemiology and Medical Ethics that are represented by 10 and 8 references, respectively. It includes rather haphazardly: Opera Omnia (80 references) and History of Medicine (182 references), various specialties, individual diseases, diseases of systems, Medical Biography (28 references), Bibliography (31 references), Lexicography (21 references), and so on. By way of further illustration, the first reference is to R. F. Harper's book (1904) *Hammurabi's Code*, the second to Joachim's (1890) *Ebers Papyrus*. The *Edwin Smith Papyrus*—the oldest of medical texts—is appropriately found under Surgery (Ref. 4734), as might be inferred from the "See also" reference to Surgery beneath the Principal Heading—"Collective Works."

To cover such a wide scope in 412 small pages obviously required strict discrimination. As Morton observes: "Much has been omitted that might have been included; it is hoped that nothing has been included which ought to have been omitted." Students of and writers on Medical History can hardly afford to be without this volume, which in view of the nature of the text will not be regarded as high priced.

E. K.

**THE DYSENTERIC DISORDERS.** The Diagnosis and Treatment of Dysentery, Sprue, Colitis and Other Diarrhoeas in General Practice. By SIR PHILIP MANSON-BAHR, C.M.G., D.S.O., M.D., F.R.C.P., Senior Physician to the Hospital for Tropical Diseases, Royal Albert Dock and Tilbury Hospitals; Consulting Physician in Tropical Diseases to the Dreadnought Seamen's Hospital, London; Director, Division of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine; Consulting Physician to the Colonial Office and Crown Agents for the Colonies; Consultant in Tropical Medicine to the Admiralty and to the Royal Air Force; Lumleian Lecturer, Royal College of Physicians, 1941. Appendix by W. JOHN MUGGLETON, M.S.M., F.I.M.L.T., Technical Assistant. Second Ed. Pp. 629; 108 illus. (some in color). Baltimore: Williams & Wilkins Company, 1943. Price, \$10.00.

SINCE December 7, 1941, there has been a general awareness in the medical profession that the so-called tropical diseases are of a great deal more significance than was formerly believed. In spite of the warnings of the medical corps of the Army and Navy, and in spite of outbreaks of dysentery, such as the one in Chicago in 1933, up to that time only an academic interest was taken in the subject by most of the medical men in the country, with the exception of those in the far South.

The second edition of this book will be received by a very much larger number of readers in the United States than was the first edition in 1939. The second edition is substantially the same as the first, but additions have been made by the inclusion of newer methods of treatment, such as the use of sulfa-guanidine in bacillary dysentery.

The simplicity and lucidity of the writing make the mass of information this book contains easily available to everyone. The illustrations are excellent.

We recommend this book to all those interested in the subject, as well as to all those who should be interested in it. E. R.

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**PAIN.** XXIII of the Association for Research in Nervous and Mental Disease. Editorial Board: HAROLD G. WOLFF, Chairman; HERBERT S. GASSER, M.D., and JOSEPH C. HINSEY, Ph.D. Pp. 468; 166 illus.; 19 tables. Baltimore: Williams & Wilkins Company, 1943. Price, \$7.50.

THIS volume is the product of 37 collaborators, who discuss 32 different topics. Some of these are: the pain threshold in man; central representation of pain; insensitivity to pain in man; the genesis of pain from the joints; pain from the bladder, ureter and kidney pelvis; pain from the pleura and pericardium; pain from the bronchi and lungs; pain from the digestive tract; pain of peptic ulcer; cardiac pain; effect of extracardiac pain on the heart; management of intractable pain by posterior rhizotomy; management of intractable pain by chordotomy; experimental studies in pain from the nasal and paranasal structures; surgical methods for relief of pain in the head and neck; headache mechanisms.

On page 242, where "scalp and neck muscles and pain" are discussed, the condition which is given several names but which would most appropriately be designated myalgic headache, is given scant and unsatisfactory consideration. As a general disorder it has been termed "an everyday affection," of which some of the local expressions are headache, stiff neck and lumbago. Its manifestations are particularly apt to appear in tendinous structures, refrigeration is often a factor in their production, and the literature on the subject is abundant. For the general condition, Gowers coined the term, "fibrositis." The disorder is best known to the Scandinavians, and anyone properly trained in the Swedish system of massage, can demonstrate the presence of these indurated muscle areas, by pressure over them with lubricated fingers and by passive stretching of the involved muscles, when sharp pain will be experienced. The Swedes not only find these "sore spots," but they have a skillful method of removing them, to the surprise and comfort of their patients.

In the discussion that followed each presentation of this Symposium of the Association, there was active participation. The Chairman of the Board must have experienced a sense of gratification, since it had been his hope that there would be a sustained effort for a better understanding of perception, leading to better management of pain as a simple sensory experience; and with additional knowledge of the autonomic, emotional and attitudinal reactions in persons of different cultural and individual experiences, the sounder should be our care of the ill.

N. Y.

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INTRODUCTION TO PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY. By L. EARLE ARNOW, PH.G., B.S., PH.D., M.B., M.D., Director of Biochemical Research, Medical Research Division, Sharp & Dohme, Inc., Glenolden, Pa.; Professor of Chemistry, Bryn Mawr College Summer School of Nursing, 1941-43. Introduction by KATHARINE J. DENSFORD, B.A., M.A., R.N., Director of the School of Nursing and Professor of Nursing, University of Minnesota. Second Ed. Pp. 574; 142 figs.; 31 tables. St. Louis: C. V. Mosby Company, 1943. Price, \$3.75.

THIS volume was written originally for use by students in nursing. Its content follows closely the outline suggested by the National League of Nursing Education (1937). Part I is a general introduction to inorganic and organic chemistry, given in sufficient detail to aid in understanding material presented later. Part II gives a discussion of physiologic and pathologic chemistry, including chapters on hormones, vitamins and nutrition. Part III is devoted to selected laboratory exercises covering most of the usual procedures along with tests for certain hormones and vitamins. Approximately 30 pages in the Appendix describe home methods for stain removal from fabrics.

Numerous descriptive diagrams and pictures of the physical changes produced by various diseases are presented along with the text. Minor changes have been made in this second edition. Newer members of the vitamin B complex, intestinal bacterial synthesis of vitamins, the sulfonamides, and transmethylation are among the newer topics discussed. Also certain clinical tests are discussed more extensively.

This text should be satisfactory for the students for which it was prepared.

H. V.

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TREATMENT OF EXPERIMENTAL DATA. By A. G. WORTHING, Professor of Physics, University of Pittsburgh; and JOSEPH GEFFNER, Weirton Steel Company. Pp. 342. New York (16): John Wiley & Sons, Inc., 1943. Price, \$4.50.

THE problems of the proper presentation of experimental data and the evaluation of data presented by others have probably, in one form or another, confronted most individuals engaged in experimental work. Many have perhaps sought vainly in the past for a single text which deals adequately with various aspects of this subject. The need for a unified presentation has been met by Worthing and Geffner's new book.

*Treatment of Experimental Data* is a concise, well-organized text. It deals systematically with such subjects as criteria for judgment of goodness of data, fit of mathematical equations, construction of tables and graphs, preferential use of equations, tables or graphs, etc. In addition to these general topics, the text affords an adequate treatment of the essentials of correlation. There is an excellent appendix upon the subject of determinants, whose value in the solution of simultaneous equations (which often crop up in biomathematics), does not appear to have been sufficiently recognized by biologists.

This text should prove of value to most graduate students, and to all workers in the field of medicine and biology who may wish to become better acquainted with the mathematical bases of treatment of experimental data. The Reviewer is glad to recommend this unique text most highly.

D. D.

**PSYCHOLOGICAL MEDICINE.** By DESMOND CURREN, M.B., F.R.C.P., D.P.M., Psychiatrist and Lecturer in Psychological Medicine, St. George's Hospital, and Honorary Psychiatrist to the Maida Vale Hospital for Nervous Diseases, London; Temp. Surgeon Captain R.N.V.R., and Consultant in Psychological Medicine in the Royal Navy; and ERIC GUTTMANN, M.D., L.R.C.P. (Ed.), Neuropsychiatric Specialist, Emergency Medical Service; Formerly Research Psychiatrist, the Maudsley Hospital, London, and Research Neuropsychiatrist, Nuffield Dept. of Surgery, Oxford. Foreword by J. J. CONYBEARE, D.M. (OXON.), F.R.C.P., Physician to Guy's Hospital, London. Pp. 188; 21 illustrations. Baltimore: Williams & Wilkins Company, 1943. Price, \$3.50.

IN this book, designed for students, general practitioners and Service physicians, the text is divided into two parts: A Short Introduction to Psychiatry, and War-time Psychiatry. In the former, most of the topics are well discussed; they are Introductory, *Ætiology of Mental Disorder*, Symptoms in Mental Disease, Psychiatric Case-taking, Treatment, Constitutional Anomalies, Organic Syndromes, Drug Addictions, Schizophrenia, Affective Reaction Types, Hysterical Reactions, and Legal Aspects of Mental Illness (since the book is published in America, inclusion of the English and Scotch laws pertaining to mental diseases, and exclusion of our own, renders that portion of the book of but little value here).

As regards psychoanalysis: "it has tended to become a special calling for the pursuit of which neither a psychiatric nor a general medical training has always been considered essential. . . . Such results as are available, do not seem to be any better than those obtained by less expensive and laborious methods." Under Intelligence Tests, no mention is made of the Rorschach procedure, which several countries have used successfully in their examinations of service men.

The part on War-time Psychiatry has but one defect—it is much too brief. There are excellent short discussions on Principles of War-time Psychiatry, Examination of Service Men, Clinical Syndromes, Management and Treatment, and Psychiatric Aspects of Head Injuries. N. Y.

**BIOCHEMISTRY FOR MEDICAL STUDENTS.** By WILLIAM VEALE THORPE, M.A. (CANTAB.), PH.D. (LOND.), Reader in Chemical Physiology, University of Birmingham. Third Ed. Pp. 476; 39 illus. Baltimore: Williams & Wilkins Company, 1943. Price, \$4.50.

EXIGENCIES of the war have prevented the author from making very many changes in the material presented in the third edition. A short chapter on the Chemistry of Respiration has been added. Rearrangement of the text has allowed the addition of some other new material.

The presentation of the general descriptive matter is both adequate in scope and detail. It is to be regretted that the weakest part of the volume is concerned with the metabolism of foodstuffs within the tissues. Most of the fundamental work of the last few years has, of necessity, been omitted. It is hoped that this (serious) omission will be corrected in any future edition. H. V.

**ELEMENTS OF MEDICAL MYCOLOGY.** By JACOB H. SWARTZ, M.D., Assistant Professor of Dermatology, Harvard Medical School and Postgraduate School, Boston, Mass., with an Introduction by FRED D. WEIDMAN, M.D., Professor of Dermatological Research, University of Pennsylvania, Philadelphia, Pa. Pp. 179; 79 figs; 1 table. New York: Grune & Stratton, Inc., 1943. Price, \$4.50.

THIS book is not a definitive text but is designed to serve the medical student or practitioner as a guide in the study of fungous diseases. Discus-

sion of certain rarer diseases has been sacrificed for simplicity and conciseness of content and expression. The book contains a general discussion of fungi and a glossary of terms which should prove helpful to the reader for orientation. One chapter is devoted to the laboratory diagnosis of fungous infections. Other chapter headings are: Blastomycetes (yeast-like fungi); Microsporium; Trichophyton; other pathogenic fungi, including Gilchrist's organisms, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, chromoblastomycetic fungi, Sporotrichum, Actinomyces, and Histoplasma. A short chapter is devoted to common contaminants and probable pathogens which may be encountered from the air. There is a very brief discussion of immune reactions (trichophytin and oidiomycin tests) and the effect on fungi of sulfanilamide and its derivatives. A chart, measuring 15 x 31 inches, gives much important information on the clinical pictures and mycologic findings for many of the important fungi. The illustrations are very good. H. M.

BIOCHEMISTRY OF THE FATTY ACIDS and Their Compounds, the Lipids. By W. R. BLOOR, Professor of Biochemistry and Pharmacology, The University of Rochester, Rochester, N. Y. American Chemical Society Monograph Series. Pp. 386; tables and charts. New York: Reinhold Publishing Corp., 1943. Price, \$6.00.

WITHIN the last few years there has developed a marked interest in the chemistry and function of the lipids. This is due in part to the development of more adequate analytical tools, and also to a greater appreciation of the ubiquitous rôle of these compounds in the life processes of all living tissues.

In this book the field of discussion is limited to those naturally occurring substances chemically and metabolically related to the fatty acids. In the preliminary chapter the descriptive and analytical chemistry of the compounds to be discussed is outlined. Subsequent chapters deal with digestion and absorption, lipids of the blood, lipids in tissue, lipid metabolism, and the lipids of secretions and excretions.

We are fortunate in having such an eminent leader in this phase of biochemistry summarize and correlate the evidence accumulated up to the present. While many phases of the subject do not permit a complete picture to be developed, this review by Dr. Bloor focuses attention upon future problems. Everyone having an interest in this phase of biochemistry will find this book of great value. H. V.

UROLOGICAL DISEASES OF PREGNANCY. By E. GRANVILLE CRABTREE, M.D., Urologist to the Boston Lying-In Hospital. With a signed chapter by GEORGE C. PRATHER, M.D., Assistant Urologist to the Boston Lying-In Hospital. Pp. 472; 158 figs., several in color. Baltimore: The Williams & Wilkins Company, 1942. Price, \$5.00.

IN the not distant past, when one thought of the urologic aspects of pregnancy, the subject of pyelonephritis of pregnancy came to mind. Other considerations were not frequently thought of. Dr. Crabtree's book is the first comprehensive treatise on the urologic aspects of the pregnant state. It represents a work of over 25 years. The information upon which the text is built could only have been obtained by extensive study of the literature and by extensive personal experience.

The great advance in the state of knowledge, concerning these subjects, which began in 1930, is recorded. The application of basic principles of urologic study to pregnant women in health and disease had to await the development of the urologic approach. This knowledge springs from the use of the modern cystoscope, retrograde pyelography, intravenous urography, renal function tests, and blood and urine chemistry.

The book contains 36 chapters and is divided into 2 parts. Among the subjects covered in the first part of the book are anatomy and physiology of

pregnancy, effect of pregnancy on preëxisting pathologic conditions in the urinary tract, and urinary tract infections. The predisposing causes of urinary infections and their treatment in the light of most recent advances are thoroughly covered. There is a separate chapter on the bladder and the effect of pregnancy, delivery, and puerperium upon it.

The technique of cystoscopy and other urologic examinations as particularly applied to the pregnant state is discussed in the second half of the book. The following subjects are discussed in detail in this section: toxemia of pregnancy, hematuria, lone kidney, tuberculosis, calculus, tumor, polycystic disease, and diabetes mellitus.

There are chapters on renal and ureteral injury in pregnancy, renal insufficiency from blood transfusion, and renal contraindications to pregnancy.

The book is well organized and profusely illustrated, and has a bibliography at the end of each chapter. It is indeed a scholarly treatise and deserves preëminence as a source book in the field.

L. La T.

### NEW BOOKS

*Backache and Sciatic Neuritis.* By PHILIP LEWIN, M.D., F.A.C.S., Associate Professor of Bone and Joint Surgery, Northwestern University Medical School; Attending Orthopedic Surgeon, Cook County Hospital; Attending Orthopedic Surgeon, Michael Reese Hospital; Professor, Orthopedic Surgery, Cook County Graduate School of Medicine, Chicago; Lieutenant Colonel, Medical Corps, U. S. Army. Pp. 745; 235 figs. Philadelphia: Lea & Febiger, 1943. Price, \$10.00.

*A Hundred Years of Medicine.* By C. D. HAAGENSEN and WYNDHAM E. B. LLOYD. Pp. 444; a few figs. New York: Sheridan House, 1943. Price, \$3.75.

*Psychosomatic Diagnosis.* By FLANDERS DUNBAR, M.D., MED.Sc.D., Ph.D., Department of Medicine and Psychiatry, Columbia University. Foreword by LEONARD G. ROWNTREE, Colonel, Medical Reserve Corps, U. S. Army. Pp. 741; no figs. New York, London: Paul B. Hoeber, Inc., 1943. Price, \$7.50.

*Medical Radiographic Technic.* Edited by GLENN W. FILES, Director of General Electric X-Ray Corporation Technical Service Department. Pp. 365; 381 figs. Springfield: Charles C Thomas, 1943. Price, \$6.00.

*The Modern Management of Colitis.* By J. ARNOLD BARGEN, M.D., M.S., F.A.C.P., Chief of the Section on Internal Diseases, Division of Medicine, Mayo Clinic; Associate Professor of Medicine, Mayo Foundation, Rochester, Minn.; Secretary, American Gastroenterological Association; Vice-Chairman, Section on Gastroenterology and Proctology, American Medical Association. Pp. 332; 148 figs. Springfield: Charles C Thomas, 1943. Price, \$7.00.

*The Arthropathies. A Handbook of Roentgen Diagnosis.* By ALFRED A. DE LORIMER, A.B., M.A., M.D., Colonel, Medical Corps, U. S. Army; Commandant, The Army School of Roentgenology, Memphis, Tenn.; Formerly Director, Department of Roentgenology, Army Medical School, Washington, D. C. Pp. 319; 678 figs. Chicago: The Year Book Publishers, Inc., 1943. Price, \$5.50.

*Medical Parasitology and Zoölogy.* By RALPH WELTY NAUSS, B.Sc., M.D., Dr.P.H., Assistant Professor of Public Health and Preventive Medicine, Cornell University Medical College; Consulting Parasitologist, New York Hospital; Fellow, American Public Health Association; Lieutenant Colonel and Flight Surgeon, Medical Reserve Corps, U. S. Army. Foreword by JOHN C. TORREY, Ph.D., Professor (Emeritus) of Epidemiology, Cornell University Medical College. Pp. 534; 95 figs. New York, London: Paul B. Hoeber, Inc., 1943. Price, \$6.00.

*A Manual of Medical Parasitology.* By CLAY G HUFF, Professor of Parasitology, University of Chicago. Pp. 88; 10 plates. Chicago: University of Chicago Press, 1943. Price, \$1.50.

*Nelson Loose-Leaf Renewal Pages—Specialties in Medical Practice.* Edited by EDGAR VAN NUYS ALLEN, M.D., Chief of a Section in the Division of Medicine, The Mayo Clinic, Rochester, Minn.; Associate Professor of Medicine, The Mayo Foundation for Medical Education and Research, Graduate School, University of Minnesota. Two Vols. Pp. 270; new front matter; 2 new color plates; revised and enlarged index. New York: Thomas Nelson & Sons, 1943.

A SERIES of revised monographs by specialists bringing such information in their respective fields, as set forth in the first edition of *Specialties in Medical Practice*, published in 1940, up to date. There is added to this first set of *Renewal Pages* an article on Minor Surgery by W. Kenneth Jennings, Pittsfield, and a revision of the article on Orthopedic Surgery, with new sections and illustrations, by Don King, San Francisco. These additions, revisions of 9 other chapters, and an enlarged index, make the 1943 edition of *Specialties in Medical Practice* especially valuable to the physician during the busy days of war-time conditions.

R. K.

### NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

MANUSCRIPTS intended for publication in the AMERICAN JOURNAL OF THE MEDICAL SCIENCES, and correspondence, should be sent to the Editor, DR. EDWARD B. KRUMHAAER, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa. Articles are accepted for publication in the AMERICAN JOURNAL OF THE MEDICAL SCIENCES exclusively, except in the case of subsequent publication in Society proceedings.

MANUSCRIPTS should be typewritten on one side of the paper only, and should be double spaced with liberal margins. The author's chief position and, when possible, the Department from which the work is produced should be indicated in the subtitle. ILLUSTRATIONS accompanying articles should be numbered and have typed captions bearing corresponding numbers. For identification they should also have the author's name written on the margin or back. The recommendations of the American Medical Association Style Book should be followed. REFERENCES should be numbered and at the end of the articles, arranged alphabetically according to the name of the first author and should be complete, that is, author's name, journal, volume, page and year (in Arabic numbers).

RETURN POSTAGE should accompany all manuscripts but will be returned to the author if the manuscript is accepted.

### NEW NOTICE TO CONTRIBUTORS AND SUBSCRIBERS

Activated by a directive from the War Production Board, we have changed the size of our type page for the "duration" to effect an economy in the amount of paper used. While there is a smaller number of pages, the amount of material has not been noticeably reduced.

We hope that any unpleasant effect produced by cutting down the margins will be accepted and approved by readers as a temporary war casualty. It is possible that more radical changes will have to be made later, but we are loath to change any more than absolutely necessary, a format that has existed practically unchanged since the Journal began in 1820.

For the balance of the war, 150 reprints will be supplied gratis. Covers will be omitted on all articles. In ordering additional reprints, we will supply in multiples of 150. This modification is for the same reason as the change of format, *i. e.*, conservation of paper.

### NOTICE TO SUBSCRIBERS AND ADVERTISERS

We desire to secure several copies of the March and May 1943 numbers of this Journal, in order to comply with requests and need for replacements in long library "runs." The war situation has made it impossible to print extra numbers to supply this demand. We would be *very* grateful to anyone who would return to the Publishers any unmutated copies of these numbers for which they have no further use, and we would be glad to repay postage.

# THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

MARCH, 1944

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## ORIGINAL ARTICLES

### THE DIAGNOSTIC VALUE OF PANCREATIC FUNCTION TESTS IN 47 SURGICALLY TREATED CASES

BY LOUIS BAUMAN, M.D.

ASSISTANT PROFESSOR OF CLINICAL MEDICINE.

AND

ALLEN O. WHIPPLE, M.D.

VALENTINE MOTT PROFESSOR OF SURGERY  
NEW YORK, N. Y.

With the technical assistance of

MISS EDITH BRATTI, A.B., and MISS PAULLYNN STINES, R.N.

(From the Departments of Medicine and Surgery, Presbyterian Hospital and  
Columbia University)

MEDICAL men have long been concerned with the motor and secretory abnormalities of the stomach. This organ furnishes pepsin and hydrochloric acid and initiates the digestion of protein. The functional disturbances of the pancreas, however, a gland which supplies protein, starch and fat-splitting ferments, have received scant attention, owing to the lesser accessibility of the organ, and to the lack of a good diagnostic test. The need for a reliable test of the external pancreatic function is obvious when one experiences the difficulty of diagnosis of purely functional disorders as well as cancer and inflammation of this organ.

Secretin, the hormonal stimulant of the pancreas, is a protein compound that can be extracted from the mucous membrane of the small intestine. We are indebted to Hammarsten and collaborators<sup>7</sup> for the preparation of a standardized secretin that is commercially available. The injection of this product into a normal person induces a copious flow of dilute pancreatic juice that is relatively poor in ferments. In acute and chronic pancreatitis Agren, Lagerlof and Berglund<sup>1</sup> found that the pancreatic juice following secretin was low in volume, bicarbonate or ferment concentration or in all 3. Diamond and Siegal<sup>15</sup> on the basis of 130 secretin tests corroborated the results of the Swedish authors and thus confirmed the clinical value of the test.

Neural stimulation of the pancreas through the vagus nerve follows the injection of methyl acetylcholine ("mecholy"). The resulting juice is more concentrated than that after secretin and therefore richer in ferments.<sup>4</sup>



In pancreatic disease, according to Diamond,<sup>6</sup> the enzyme content of the juice is affected before the bicarbonate concentration. For this reason and because of the difficulty of procuring secretin at the present time our experiences are limited to the use of mecholyl.

The limitations of all functional tests are appreciated. A normal bromsulphalein liver function test is possible when the organ is riddled with carcinoma, provided that normal intervening parenchyma is present. The same applies to kidney tests. The remaining islets suffice to maintain normal dextrose utilization when three-quarters of the human pancreas is ablated.<sup>9</sup> The truism that no test is 100% reliable and that laboratory results must be correlated with the clinical picture hardly need repetition.

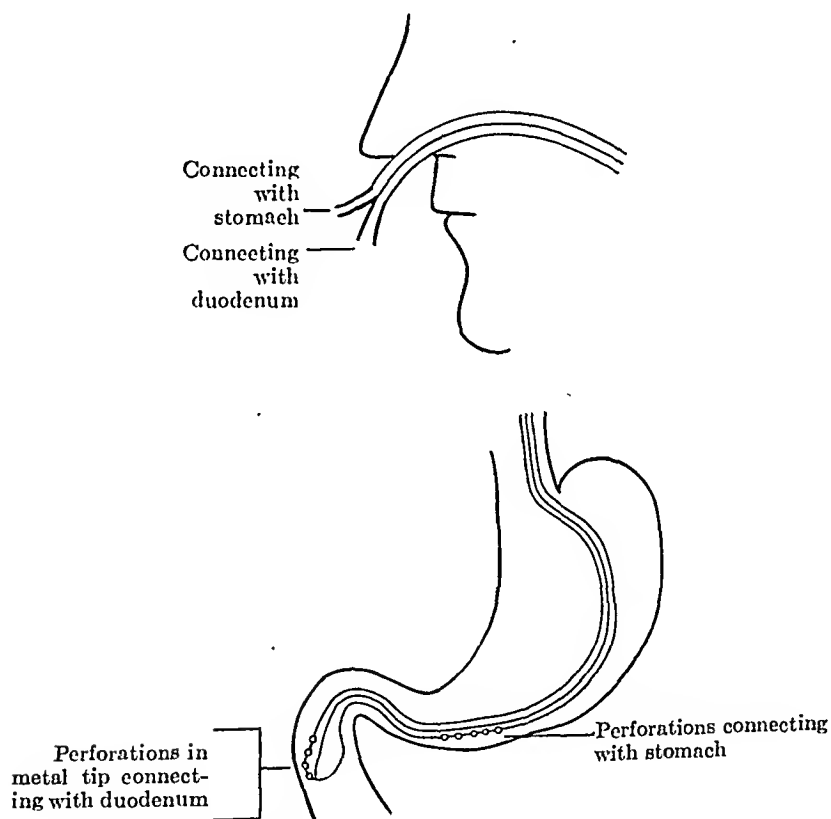


FIG. 1.—Diagrammatic illustration of removing substance from pancreas.

That disturbance of physiology is not always associated with gross or even microscopic changes is becoming clearer as functional organ studies progress. To quote Professor Virginia Kneeland Frantz,\* "In both benign and malignant tumors of islet cells there are no histologic criteria by which we can distinguish those which are functional and those which are not. With an exceedingly small adenoma, a patient may be unconscious from hypoglycemia while with an even larger

\* Personal communication.

similar tumor in another case there may be no symptoms of hyperinsulinism."

Recently a middle-aged man with prolonged digestive disturbance and distress in the upper abdomen had normal gall bladder and stomach Roentgen ray reports. He also had achylia gastrica and deficiency of pancreatic ferments yet histologic examination of part of the pancreas removed at operation was normal. It is important to realize that absence or diminution of ferments in the pancreatic juice does not itself signify organic disease of this organ.

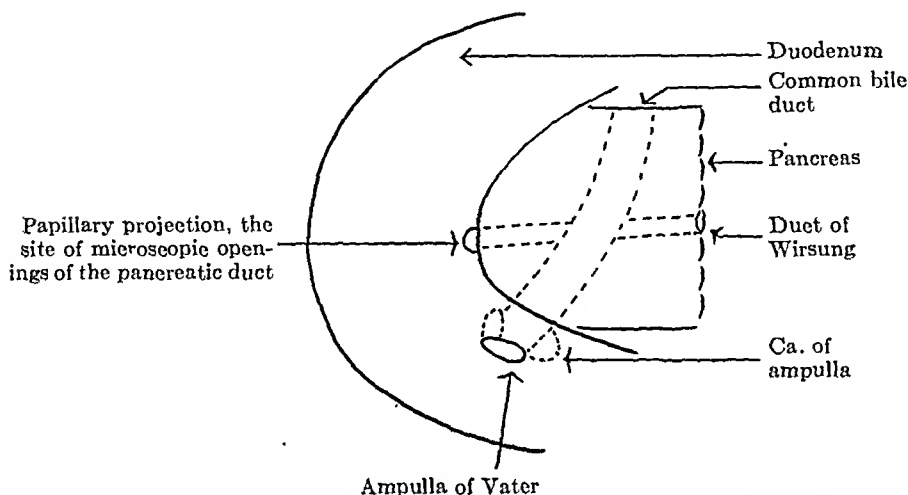


FIG. 2.—Diagram of anatomy of specimen removed at operation. (Case 686471.)

*Technique.* A rubber tube 4 feet long and 16 French in diameter is used. It is divided into two compartments throughout its length by a central longitudinal partition. One end is provided with a perforated metal tip, the other end projects from the nose where each canal is connected with a short tube. One compartment is perforated at that part of the tube which is in contact with the body of the stomach but has no connection with the duodenum; the other canal is only connected with the duodenum (Fig. 1). The gastric and duodenal secretions can therefore be separately removed by gentle aspiration. Acidification with hydrochloric acid destroys the pancreatic ferments.

After the tube has been passed into the stomach through the nose, the patient is placed on the right side and transported to the fluoroscopy room. The assistance of the roentgenologist\* is necessary to manipulate the tube into the duodenum. This is often difficult, time-consuming and at times unsuccessful. Pylorospasm or abnormal conformation of the stomach or duodenum may be the cause of failure. Fifteen mg. of mecholyl are now injected subcutaneously and after 10 minutes the first fraction of pancreatic juice is obtained. Three further 10-minute fractions are collected. Untoward mecholyl effects can be counteracted by atropine. To avoid deterioration of ferments the juice should be collected in tubes immersed in ice-water. If the pH of the fractions is above 7 their ferment activity is determined by the following procedures: Amylase and protease by modified Wohlgemuth and Gross casein methods, respectively,<sup>2</sup> and the lipase by the Cherry and Crandall method.<sup>3</sup>

\* We are indebted to Dr. Ross Golden and his staff of the Roentgenology Department of the Presbyterian Hospital for their help.

TABLE 1.—NORMAL FERMENT CONCENTRATIONS OBTAINED IN VARIOUS DISEASES

Hist. No.	Date, 1941	Reaction pH	Fraction	Ferments				Serum amylase	Operation	Diagnosis and remarks
				A	L	P				
563206	...	8.0	a.	+	+	+	+	20	Yes	Stones in gall bladder. Pancreas looked normal. Typical gall stone history. Pancreas showed no evidence of past or present inflammation. Gall bladder filled with stones was removed.
449451	10/2	8.0	c.	+	+	+	+	..	Yes	Chr. cholecystitis. Stones in gall bladder. Pancreas not explored.
657331	10/29	8.0	a.	+	+	+	+	22	Yes	Gall stones. Stone in common bile duct above ampulla 3.6 cm. Pancreas mostly normal histologically. Some scar tissue about acini. Pain in epigastrium and right upper quadrant. Jaundiced. Cholesterol 685. Bilirubin 10. Phosphatase 7. At operation stone in gall bladder and common bile duct.
664298	1/8/42	7.5+	a.	+	+	+	+	..	Yes	Painless jaundice with normal ferments. Ca. of common bile duct proximal to ampulla of Vater. Gall bladder palpable. Phosphatase 12. Bilirubin 24. Cholesterol 585. Growth involved lower third of common bile duct but not ampulla.
508263	1/19/42	7.5	a.	+	+	+	+	39	Yes	Stones in common bile duct. Pancreas felt normal at operation. Man; 60 yrs. Several attacks. Right upper quadrant, pain with jaundice. Bilirubin 9. Amylase 60. Protease 4. At operation—stones in gall bladder and common bile duct. Pancreas slightly edematous. Otherwise normal.
624297	10/22/41	7.7	a.	+	+	+	+	..	No	Undiagnosed. Normal control.
656238	10/28/41	8.0	a.	+	+	+	+	..	Yes	Kidney stone. Normal control.
660772	11/27/41	7.2	a.	+	+	+	+	..	Yes	Gall stones.
638191	4/18/41	7.5	b.	+	+	+	+	112* 31*	Yes	Chr. cholecystitis. Stones in gall bladder. Pancreas firm and almost nodular. Diabetic curve. Woman; 56 yrs. Long history of indigestion and epigastric pain radiating to shoulder blade. Two weeks ago last attack much more severe. Fever. Leukocytosis. Traces of serum bilirubin. No jaundice. Tenderness in mid-epigastrium. Cholecystogram showed non-filling gall bladder. At operation, gall bladder distended and edematous. No stones in common duct. Pancreas enlarged, firm and almost nodular. Liver normal.
658890	1/7/42	7.5	a.	+	+	+	+	..	No	Undiagnosed condition of abdomen.
NORMAL PANCREATIC JUICE										
Hist. No.	Reaction of each fraction	Ferments				Serum amylase	Operation	Diagnosis and remarks		
		A	L	P						
676458	+7.5	+	+	+	+	0	Yes	Painless jaundice (obstructive). Inflamm. of common bile duct and cirrhosis. A series of pancreas histology = normal. Man; 33 yrs. Jaundice (painless). Enlarged liver and spleen. Bilirubin 11.5. Phosphatase 20. Operation, cirrhosis-ascites. Gall bladder not distended; common bile duct not dilated. Induration head of pancreas, but biopsy normal. Normal pancreas by biopsy. Woman; 47 yrs.; 3 yrs. history of jaundice. Had large liver and spleen. Was anemic, leukopenic, low leukocyte count. Phosphatase 37. Bilirubin 9. Cephalin test, 4+. At oper. liver enlarged, appearance of biliary cirrhosis. Gall bladder small. A hard mass either in second part of duodenum or pancreas. Biopsy of liver: biliary cirrhosis; hard mass; normal pancreas.		
692568	+7.5 +7.5 +7.5 6.3	+	low	+	+	..	Yes	Ca. common bile duct verified by biopsy. Female; 47 yrs. Epigastric fullness (10 weeks). Jaundiced. Cholecystostomy. Jaundice continued. Enlarged liver. Glucose tolerance normal. Phosphatase 38.2. Cephalin 0. Bilirubin 12.1. Amylase 12. At operation: ca. of common bile duct involving cystic duct.		
689707	+7.5 +7.5 +7.5	+	+	+	+	11	Yes	Ca. bile duct. Female; 52 yrs. Jaundice. Diarrhea. Mass in right upper quadrant. Phosphatase 6.6. Bilirubin 5. Operation: liver enlarged, gall bladder distended; hard mass at junction common and cystic extending up into common hepatic duct and into portal fissure.		
685484	+7.5 +7.5 +6.8	+	+	+	+	..	Yes			



TABLE 2.—VERIFIED PANCREATIC DISEASE

Hist. No.	Reaction of each fraction	Ferments				Serum amylase	Operation	Diagnosis and Remarks
		A	L	P				
639299	7.0+	+	0	0	0	37	Yes	Ca. of pancreas. Male; 37 yrs. Upper abdominal pain and tenderness. Jaundice. Cholecystojejunostomy. Distended gall bladder. Enlarged pancreas. After operation pain continued. Fatty diarrhea. Glucose tolerance normal. Second operation: hard, firm mass in head of pancreas. Histology: cancer.
635632	9.46	0	+	+	+	228	Yes	Chr. cholecystitis. Stones in gall bladder. Pancreas enlarged, thickened and indurated. No stones in common duct. Female; 34 yrs. Long history of severe epigastric pain radiating to back. No jaundice. Tenderness over epigastrium. High amylase.
652693	7.6	+	0	0	0	..	Yes	Ca. of pancreas. Male; 68 yrs. Jaundiced. Diarrhea. Bilirubin 20. Phosphatase 25. Operation: growth of head and body of pancreas. Gall bladder distended.
657473	7.0	low	+	+	+	44	Yes	Adenoma of islands of Langerhans. Head of pancreas, possibly pressing on pancreatic duct. Male; 49 yrs. Intermittent loss of consciousness. Vertigo. Low blood sugars. Operation: tumor head of pancreas near the common duct. Histology: adenoma or cancer of islands of Langerhans.
697965	5.2	+	low	+	+	..	Yes	Ca. of pancreas. Male; 60 yrs. One year of fullness of abdomen. Ache around umbilicus. Appetite poor. Jaundiced 3 weeks. Liver and gall bladder palpable. Bilirubin 12.7. Phosphatase 11.7. Cholesterol 437. Operation: prepyloric mass involving common duct and pancreas; Autopsy: ca. head of pancreas with ulceration in stomach and duodenum.
657368	8.0+	+	low	+	+	22	Yes	Chr. pancreatitis at operation associated with gall stones. Female; 55 yrs. Painless jaundice. Bilirubin 16. Amylase 22. Phosphatase 17. Stones in gall bladder. Common duct enlarged. Head of pancreas showed patchy thickening. Biopsy: pancreaticitis.
700227	7.5+	+	low	0	0	..	Yes	Ca. head of pancreas involving common bile duct. Male; 73 yrs. Painless jaundice. Large liver. Bilirubin 13. Phosphatase 67. At operation: enlarged gall bladder, large liver. Autopsy: ca. of head of pancreas.
678265	+7.5 +7.5 +7.5 +7.5 +7.5	low low low low low	0 +	low +	low +	70	Yes	Chronic pancreatitis. Pancreas hard and enlarged. Bilirubin 8.3. Female; 46 yrs. Right upper quadrant pain. Non-filling gall bladder. Later pain in left upper quadrant radiating to back. Jaundiced. W.B.C. 16,100. Bilirubin 8.3. Amylase 70. Operation: stones in gall bladder. Pancreas large and hard.
692078	+7.5 +7.5 +7.5 +7.5	low low low low	+	+	+	88	Yes	Infected necrotic cyst of pancreas. Diabetic glucose tolerance curve. Ferments in cyst fluid. Female; 40 yrs. Two months prior to admission epigastric distress, vomiting. Later epigastric pain. Rigidity of epigastrium and tenderness. High serum amylase. Mass in abdomen. Temperature up to 102°. Elevated W.B.C. No jaundice. Roentgen ray: displacement of stomach and duodenum due to enlarged head of pancreas.
687251	4.5 6.3 5.2 5.0 6.0 7.5 +7.5 7.0	0 0 0 0 0 0 0 0	low +	0 low low low low low +	0 low low low low low +	14.5	Yes	Ca. pancreas verified by histologic examination. Fluid contained amylase and lipase. 39 yrs. Three months pain in back, region of left kidney requiring morphine. Amylase 15. Phosphatase 3.9. No jaundice.
438079		0 0 0 0 0 0 0	low low low low low low +	0 low low low low low +	0 low low low low low +	22	Yes	Chronic pancreatitis confirmed histology. Is a diabetic. Abdominal pain left mid-abdomen. Epigastric fullness, weight loss, slight tenderness right upper quadrant. Bilirubin 6. Phosphatase 20. Amylase 22. At operation gall bladder tense and distended. Large hard pancreas. Section: fibrosis. Repeated pancreatic function test in February 1943 and found no ferments in juice.

680814	4.6 +7.5 +7.5 +7.5 +7.5	0 + low low low	- + low low low	24	Yes	Chronic pancreatitis. Diabetic glucose tolerance curve. Female; 66 yrs. Belching 2 yrs. Cholecystogram: abnormal. Now jaundice, chills, fever, enlarged liver. Bilirubin 13.8. Phosphatase 17.1. Diabetic glucose tolerance curve. Operation: distended gall bladder. Pancreas hard and nodular.
430645	7.0 +7.5	low low	low low	..	Yes	Chronic pancreatitis. Stones in gall bladder and common duct. Died of cancer of liver, metastatic. Pancreas not explored. Had been operated upon by Dr. Whipple for gall stones and cholangitis, stones in common bile duct. Male; 33 yrs. Followed for several years. Chronic pancreatitis. Nodular body and tail. Male; 33 yrs. Followed for several years. Routs of pain left upper quadrant. Fever and pleurisy with effusion (left). W.B.C. 8600. Op. elsewhere. Calcified abdominal nodes removed. Operation: nodular tissue behind April 1939, gall bladder removed. Blood amylase 43. Follow-up: doing well at present time. Pancreas, probably chronic pancreatitis. Follow-up: doing well at present time. Jaundice. Enlarged liver. Phosphatase 23. Bilirubin 8.3. Cholesterol 335. Narrowing of duodenal bulb. Operation: ca. pancreas involving head and body.
679477	-4.5 7.5 -4.5	0 + 0	0 low low	..	Yes	Acute pancreatitis. Head of pancreas firm. Diabetes. Bilirubin 9.9. Test done 52 days after operation in O.P.D. Diabetic male; 42 yrs. Severe upper abdominal pain chiefly epigastric lasting 5 days and associated with vomiting. Little tenderness but spasm right upper quadrant. Bilirubin 9.9. W.B.C. 12,500. Fever. Diagnosis: acute cholecystitis, stones in common duct. At operation gall bladder distended (100 cc.). No stones in gall bladder or common duct. Edema of common duct with firm head of pancreas. Path. report: acute cholecystitis. Amylase postoper. 146.
667695	+7.5 +7.5	low +	low +	146	Yes	Ca. pancreas confirmed by histologic examination. Bilirubin 12. Phosphatase 11.4. Male; 60 yrs. Pain in back level 12 T vertebra. Later jaundiced. Enlarged liver. Cholecystectomy at another hospital. Diarrhea. Bilirubin 10.6. Phosphatase 7.6. Operation: induration head of pancreas. Biopsy: ca.
675759	-4.5 +7.5 -4.5	0 + 0	low low low	30	Yes	Ca. head of pancreas without jaundice. Male; 83 yrs. Vomiting for 1 yr. Roentgen ray: obstruction third part of duodenum. Operation: ea. head of pancreas without jaundice.
686760	+7.5 +7.5 +7.5 +7.5 +7.5	low low low low low	low low low low low	..	Yes	Cyst attached to tail of pancreas. Biopsy: neuroblastoma. Glucose tolerance normal. Cyst (in left abdomen, below costal margin). Arose from region of tail of pancreas, adherent to aorta. Pathologic diagnosis: neuroblastoma.
689412	+7.5 +7.5 +7.5 +7.5 +7.5 +7.5 +7.5	low low + + + + +	low low low low low low low	33	Yes	Following cholecystectomy for gall stones. Five years of attacks epigastric pain radiating to back. Cholecystectomy 16 years ago for gall stones. Pancreas not explored. One week after operation epigastric pain radiating to left shoulder. Nine, 8 and 7 years ago bouts of epigastric pain. On admission, epigastric pain, vomiting, weight loss. Bilirubin 6.4. Amylase 53. Phosphatase 11. Is being followed without op. Diagnosis: chronic pancreatitis. Diabetic glucose tolerance test.
678490	+6.0 +7.5 +7.5 +7.5 +7.5	low low low low low	low low low low low	53	No	Chronic pancreatitis. Induration head pancreas. Male; 38 yrs. In 1937, crampy epigastric pain radiating to back with 2 subsequent attacks, the last with fever, epigastric rigidity and tenderness. W.B.C. 28,000. Amylase 73. Bilirubin 5. At operation: stones in gall bladder and common duct. Pancreas large and firm. Cholecystectomy and drainage of common bile duct. 8/29, attacks of epigastric pain through to back. Tender L. epig. Amylase 95. Bilirubin 1.4/40. L.U.Q. pain. 11/40, readmission, R. and L.U.Q. pain. Operation: indurated head of pancreas. Cholecystocholecystectomy. No stones. 4/41, recurrence of pain R.U.Q. 1/42, amylose 49. Operation: hard lumps in pancreas. Plastic on ampulla. 10/42, recurrence of attacks.
543316	-4.5 7.5 6.4 5.0	low low low low	- + + +	48.7	Yes	

TABLE 2.—VERIFIED PANCREATIC DISEASE—Continued

Hist. No.	Reaction of each fraction	Ferments			Serum amylase	Operation	Diagnosis and Remarks
		A	L	P			
668231	5.0	low	low	+	25	Yes	Ca. of pancreas. Weight loss. Vomiting. Sharp lower abdominal pain for 3 mos. Phosphate 9. No jaundice. Operation: ca. pancreas with liver involvement.
	6.9	0	+	low			
	6.9	0	0	low			
	4.5	0	0	low			
673028	4.5	0	low	0	..	Yes	Chronic pancreatitis. Ca. of pancreas. Glucose tolerance diabetic. Male; 56 yrs. 7/1/41, sharp epigastric pain. Later jaundice. Bilirubin 4. No anemia. (1) Operation (8/7/41): distended normal gall bladder. Entire pancreas enlarged, hard and fibrous. Pathologic report: fibrosis. Cholecystojejunostomy. (2) Operation 4/13/42: choledochojejunostomy-cholecystectomy. Head and body of pancreas swollen and hard. Two large sections showed only fibrosis. Prev. cholecystojejunostomy was not functioning. He absorbed 86% of ingested fat. Pain severe, continued. (3) Operation 8/3/42: revealed ca. of ampulla. Probable ca. of ampulla with pancreatitis.
	7.5	low	low	0			
	7.5	low	low	0			
	-4.5	0	0	0			
671069	-4.5	0	0	0	..	Yes	Ca. of pancreas. Male; 76 yrs. Six to 8 months mild epigastric pain and fullness, anorexia, jaundice. Mass in right upper quadrant. Bilirubin 22. Phosphatase 14. Cholesterol 369. Operation: large indurated mass involving head of pancreas. Distended gall bladder.
	-4.5	0	0	+			
	-4.5	0	0	+			
	-4.5	0	0	+			
672081	7.2	low	low	low	31	Yes	Marked fibrosis and calcification. Diabetic glucose tolerance curve. Chronic pancreatitis in Roentgen ray with calcification seen. Male; 48 yrs. Five to 6 years of abdominal pain going through to back. One attack associated with jaundice. Operation: cholecystectomy of a gangrenous inflamed gall bladder. Recurrence of pain. Roentgen ray showed calcification of the pancreas. Diabetic glucose tolerance curve. Pancreatic duct sewed into jejunum. Good follow-up January 9, 1943.
	7.5	low	low	low			
	7.3	low	low	low			
	-4.5	0	low	0			
671642	7.5	0	+	+	61	Yes	Chronic pancreatitis with stones in pancreatic duct. Male; 42 yrs. Vague digestive symptoms. Pain in epigastrium through to back. Abdominal distention. Cholecystogram, barium enema, G.-I. series negative. During attack spasm mid-epigastrium. Elevated W.B.C. Amylase 49. No abnormal calcification in region of pancreas. Amylase 61. No jaundice. Operation: no distention of gall bladder or common bile duct. Hard edematous head of pancreas thought to be malignant. Calcification when cut. Histology: pancreatitis, pancreaticolithiasis. Diabetic glucose tolerance curve. Follow-up 2/3/43: doing well.
	7.5	0	+	+			
	7.5	0	+	low			
	7.5	0	+	low			
701945	-4.5	0	+	0	54	Yes	Pancreatic involvement by perforated peptic ulcer. Male; 56 yrs. Seven years of pain in right upper quadrant, epigastrium and lower abdomen radiating to chest and back. Occasional vomiting. Cholecystogram: normal. G.-I. series: ulcer pylorus. At operation pyloric or duodenal ulcer perforating into the head of pancreas.
	7.5	+	low	0			
	7.5	+	low	0			
	5.0	low	+	+			

**Results.** We have divided the cases into 2 groups, those with normal ferment concentrations and those with abnormal ferment concentrations due to proven pancreatic disease.

*Normal Results* (Table 1). It is apparent that in cases of stone in or cancer of the bile ducts, normal pancreatic juice is usually obtained. Painless obstructive jaundice associated with normal pancreatic juice is usually found in cancer of the bile ducts. In tumor of the ampulla normal pancreatic ferments may be obtained if the pancreatic duct or ducts enter the duodenum separately, or if there is an accessory duct of Santorini.

Figure 2 is a diagram of the anatomy of the specimen removed at operation from Patient 686471. Grossly the tumor at the ampulla was easily demonstrable but the entrance of the pancreatic duct could not be found though normal pancreatic ferments had been obtained. Microscopic examination revealed minute branches of the duct of Wirsung entering the duodenum.

In only 1 patient (No. 671580) with proven cancer of the pancreas were normal ferments obtained. In this case there was a cancer of the head of the pancreas invading the common bile duct without obstructing the pancreatic duct.

In the series with normal pancreatic ferments no evidence of pancreatic disease was found except in Case 657331, where the histology of a biopsy specimen showed a predominantly normal picture with an occasional patch of fibrosis.

Patient 638191 had a serum amylase that was elevated to about 3 times the normal level, a diabetic glucose tolerance curve but normal concentrations of ferments in the pancreatic juice. The surgeon found the pancreas firm and almost nodular to palpation.\* The serum amylase tests preceded the pancreatic function test by about 2 weeks.

Patient 508263 with stones in the common duct had been observed for 5 years but had steadfastly refused operation before. The possibility of pancreatitis was considered but with normal ferments this supposition was considered unlikely. At operation 7 stones were found in the common duct. The pancreas felt normal.

*Results in Pancreatic Diseases* (Table 2). Painless obstructive jaundice with low or absent pancreatic ferment concentration is almost always due to cancer of the pancreas. Pancreatitis is usually associated with pain and if acute an elevated serum amylase is the rule. The determination of serum amylase has become one of the important clinical laboratory procedures. An elevated serum amylase is a reliable indication of acute pancreatic disease. This ferment is readily excreted by the kidney so that a fleeting edema of the pancreas may escape recognition if the test is delayed.

It is noteworthy that alkaline juice is usually present even in advanced pancreatic disease and that the activity of the 3 ferments is not affected to the same extent. In some patients the concentration of 1 ferment remains normal while that of the other 2 is markedly diminished.

\* Deductions drawn from the feel of the pancreas at operation have proven erroneous in a number of cases.



**Summary.** The reaction and ferment concentration of the pancreatic juice has been determined in about 150 patients.

This experience, together with that of Pratt<sup>8</sup> of Boston and the other clinicians already quoted, indicates that a test of the external function of the pancreas may well become an important diagnostic procedure.

**Conclusions.** Mecholyl injection induces the secretion of a relatively concentrated pancreatic juice which is well adapted for ferment determination.

Separate aspiration of the stomach is necessary to avoid acidification which injures the pancreatic ferments. A tube with 2 lumina is used for this purpose.

Functional disturbances of the external secretion of the pancreas may occur without demonstrable histologic changes in the organ.

Painless obstructive jaundice with normal pancreatic ferments favors the diagnosis of carcinoma of the bile duct. In tumor of the ampulla, normal ferments may be obtained if the pancreatic duct enters the duodenum separately or if there is an accessory duct of Santorini.

Painless obstructive jaundice with diminished or absent ferments favors the diagnosis of carcinoma of the pancreas.

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### THE EFFECTS ON EXPERIMENTAL TUBERCULOSIS OF 4,4'-DIAMINODIPHENYLSULFONE

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At the present time the chemical agents that have proved most effective in combating experimental tuberculosis of guinea pigs have been derivatives of 4,4'-diaminodiphenylsulfone.<sup>1,4,7,8,13,15</sup> Although a fairly large number of derivatives of this compound have been subjected to tests *in vivo* to determine their possible effects on tuberculosis

induced experimentally, relatively few experiments have been reported concerning the ability of 4,4'-diaminodiphenylsulfone to influence an experimental tuberculous infection.

Rist, Bloch and Hamon,<sup>14</sup> in 1940, reported on the effect of 4,4'-diaminodiphenylsulfone on rabbits and guinea pigs inoculated intravenously with avian tubercle bacilli. Since peroral administration of the drug in the dose employed induced in rabbits considerable and serious toxic manifestations, the rabbits received the drug subcutaneously suspended in olive oil. The drug, mixed with mucilage of tragacanth and syrup, was given by mouth to the guinea pigs. Although the experiments were of relatively short duration the results, based on the number of tubercle bacilli in the lesions and the histologic character of the tissue changes, indicated that a definite inhibitory effect had been achieved.

In 1942 Smith, Emmart and Westfall<sup>15</sup> studied the effects of several compounds, including 4,4'-diaminodiphenylsulfone, on experimental tuberculosis of the guinea pig. Although histopathologic studies were not reported, they stated that of the several drugs that exerted a deterrent effect on experimental tuberculous infection 4,4'-diaminodiphenylsulfone was the most effective. Smith, Emmart and Westfall emphasized the toxic nature of this drug but in view of its high specificity they expressed the belief that the search for more effective and less toxic derivatives should provide a promising field of investigation.

**The Drug.** 4,4'-Diaminodiphenylsulfone is a drug of unusual interest and importance because of the possibility that some previously reported tuberculotherapeutic drugs ("promin"\* and diasone† may break down in the body to diaminodiphenylsulfone or a similar substance common to all. There is no proof that such breakdown occurs, but the union between the parent substance and the added radicals would appear to be unstable. Experience has shown that promin has quite different effects when given orally than it has when given parenterally, suggesting that some significant chemical transformation occurs in the digestive tract.

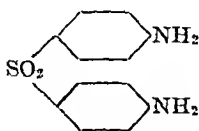


FIG. 1.—Structural formula of 4,4'-diaminodiphenylsulfone.

4,4'-Diaminodiphenylsulfone, which we obtained through the kindness of Dr. E. A. Sharp and Dr. L. A. Sweet, Parke, Davis & Co., Detroit, Mich., is somewhat simpler in chemical constitution than are most of the more popular chemotherapeutic compounds. The structural formula assigned to this compound is illustrated in Figure 1. As may be noted, 4,4'-diaminodiphenylsulfone is not a sulfonamide

\* Sodium p,p'-diaminodiphenylsulfone-N,N'-didextrose sulfonate.

† Sodium formaldehyde sulfoxylate diaminodiphenylsulfone.

derivative, as are most of the widely used products available at this time. The diphenylsulfone nucleus of this substance is the essential feature of the so-called "sulfone" group of compounds which sharply distinguishes them from the "sulfonamide" group of compounds. It is important to emphasize the distinction between these 2 groups of substances because of increasing evidence that the therapeutic and toxic effects are quite different and contrasting in the 2 instances. Significant tuberculo-therapeutic effects are essentially lacking in the sulfonamide series but are present in striking degree in several compounds of the sulfone series. There are differences in the toxic effects of the 2 groups of substances and their absorption, excretion, detoxication and mode of action may be quite different, but the latter have been inadequately studied.

Diaminodiphenylsulfone, which has a molecular weight of 248, is quite insoluble in water at room temperature (0.01%). Its crystals are described as long, rectangular, colorless flakes that have a melting point of 176° C. It has little taste and may be mixed with the feed of animals without impairing palatability. In mice, toxic doses of the drug cause the animals to become agitated and restless in behavior, undoubtedly a neurotoxic effect. Incidentally, sulfanilamide is also a neurotoxic drug for mice but produces paralysis rather than excitement and stimulation. Diaminodiphenylsulfone is absorbed rather slowly from the gastro-intestinal tract, and the presence of the compound in the blood stream persists for a much longer period than in the case of sulfanilamide and related substances. Long and Bliss<sup>12</sup> stated that "its acute toxicity is so great as to preclude its use in clinical therapeutics." However, Long and Bliss did not present the evidence on which this conclusion was based. No adequate study of the effects of this compound on the human being has come to our attention.

4,4'-Diaminodiphenylsulfone was first synthesized by Fromm and Wittmann<sup>11</sup> in 1908, but the drug did not find any practical application until 1937 when Buttle, Stephenson, Smith, Dewing and Foster<sup>3</sup> discovered its remarkable efficacy in treatment of experimental streptococcal infections of mice. They noted that this compound was much more effective than sulfanilamide in curing streptococcal infections of mice but likewise it was more toxic. It was much less toxic for rabbits and monkeys than for mice but produced methemoglobin more readily than did sulfanilamide. Buttle and his associates found that when a single dose of 0.3 gm. was given to a man his blood became bactericidal for streptococci. Fournau, Tréfouël, Nitti, Bovet and Tréfouël<sup>9</sup> made similar observations while working independently with this drug at the same time.

Bauer and Rosenthal,<sup>2</sup> in 1938, reported that 4,4'-diaminodiphenylsulfone was about 30 times as effective as sulfanilamide against streptococci. They found the therapeutic index\* to be 6, compared with an index of 3.3 for sulfanilamide and 20 for diacetyldiaminodiphenylsulfone. No extensive effort at clinical application of these findings

\* The therapeutic index was determined by Bauer and Rosenthal<sup>2</sup> by dividing the maximal tolerated dose by the minimal effective dose.

had been reported, doubtless because of the toxic potentialities of the drug.

4,4'-Diaminodiphenylsulfone has also been found to be distinctly superior to sulfanilamide in curing mice of pneumococcal infections.<sup>3,10</sup>

**Methods.** The report that follows is based on data from 3 separate experiments. In all instances young adult male guinea pigs whose average weight was approximately 450 gm. were used. The feed consisted of so-called rabbit chow to which was added corn syrup supplemented twice weekly with fresh cabbage. The infecting agent was a human tubercle bacillus, strain H37RV, grown on the synthetic medium of Proskauer and Beck. The animals were caged in pairs. The drug was mixed with the feed to the amount of 0.33% by weight.\* The daily intake of the drug was estimated to be approximately 150 mg. per animal.

*Experiment 1.* Twenty-eight animals were utilized. These were divided into 2 groups of 14 guinea pigs each. Two days before the animals were inoculated with tubercle bacilli the drug was added to the feed of 1 group. Forty-eight hours later each animal in both groups was inoculated subcutaneously in the sternal region with 0.1 mg. of an 18-day-old culture of tubercle bacilli (H37RV). Administration of the drug with the feed was continued daily, and the experiment was terminated on the 60th day after infection.†

*Experiment 2.* Two groups of 10 guinea pigs each were selected. The experimental procedure followed was essentially the same as that described for experiment 1.‡ The experiment was terminated 60 days after inoculation with tubercle bacilli.

*Experiment 3.* Forty-two guinea pigs were used. Each was inoculated subcutaneously in the sternal region with 0.0005 mg. of a 23-day-old culture of H37RV.

Forty-two days later all of the guinea pigs were found to be sensitized to tuberculin.§ At this time the animals were divided into 2 groups. Group 1 consisted of 28 animals; these were the untreated controls. Group 2 consisted of 14 animals; these were treated with 4,4'-diaminodiphenylsulfone. (Treatment was thus begun 6 weeks after the animals received the infective dose.) The experiment was continued for 228 days after the animals had received the infective inoculum. The animals in the treated group had received the drug for a total of 186 days. When the experiment was terminated, the animals that were living, in both groups, were killed.

**Results.** *Comparative Survival Times.* In Experiment 1, in which there were 14 animals in each group (treated and untreated), only 1 guinea pig had died when the experiment was terminated on the 60th day. The 1 death occurred in the group that was being treated. The animal died 35 days after inoculation with tubercle bacilli and had received 4,4'-diaminodiphenylsulfone for a total of 37 days. The cause of death was not determined. Since only 1 animal in the treated group died and all of the untreated ones lived, the difference in the survival time of the animals in the 2 groups appears to be of no significance.

\* This dose was selected after feeding trials and represents an approximate molecular equivalent to 3 times as much promin. The molecular weight of promin is 780 while that of 4,4'-diaminodiphenylsulfone is 248.

† Details concerning the feeding of guinea pigs in experiments pertaining to chemotherapy of tuberculosis and information regarding the strain of tubercle bacilli used (H37RV) and its cultivation have been published previously.<sup>7</sup>

‡ In Experiment 2, the age of the culture was 20 days.

§ Gilleland's OT diluted 1:100. Each animal received 0.02 to 0.03 cc. intracutaneously.

In Experiment 2, in which there were 10 animals in each of the 2 groups, 2 guinea pigs died during the 60-day period of observation. Both animals that died belonged in the group that was being treated. The first animal died on the 22nd day and the second on the 55th day after inoculation. In each instance the cause of death was not apparent.

In the third experiment 20 of the 28 (71.4%) animals in the untreated group died before the end of the observation period (228 days after infection) while in the group that had been treated 10 of 14 (71.4%) were living 228 days after inoculation (Fig. 2). The first death among the untreated controls occurred on the 92nd day after

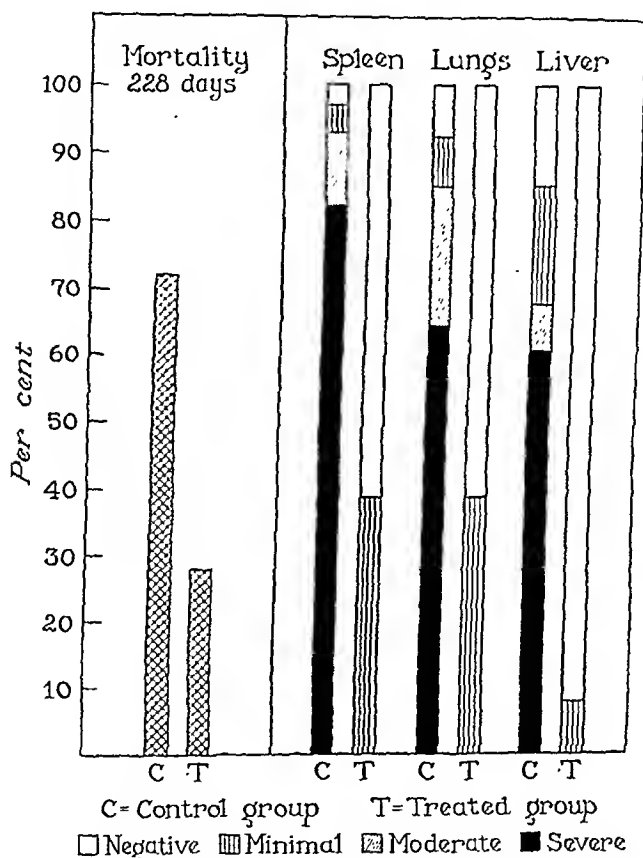


FIG. 2.—Relative mortality rates and amounts and character of tuberculosis in treated and in untreated guinea pigs (Experiment 3).

infection and with one possible exception each of the 20 animals in this group that died had a sufficient amount of tuberculosis to have been responsible for death. The 4 deaths among the animals that were receiving 4,4'-diaminodiphenylsulfone occurred on the 47th, 78th, 88th and 111th day after infection, respectively. Tuberculosis was the obvious cause of death of the animal that died on the 47th day. The disease was severe in the spleen and lungs and moderate in the liver. This animal had received treatment for 5 days only. The cause of death of the other 3 animals in the treated group that died before the experiment was terminated was not determined. Each of the 3

guinea pigs was tuberculous, but the disease was minimal and presumably not sufficient to account for death.

In the first 2 or short-term experiments, since none of the controls died during the 60-day period of observation and 3 deaths occurred among the animals receiving 4,4'-diaminodiphenylsulfone, one may presume that the animals died from the toxic effects of the drug. This is a presumption only, since objective signs indicative of toxicity were not observed. From the point of view of therapeutic efficacy the mortality rate is of no value in an experiment of 60 days duration when the infective agent is introduced into the subcutaneous tissues. Even though excessively large amounts of the infective inoculum be used, 60 days is too short a period to expect any considerable number of untreated controls to die. In a short-term experiment evidence of therapeutic effect must be sought in the amount and character of the tuberculosis in the respective animals.

In the third experiment the observations were continued for a sufficiently long period to make the relative mortality rates of the treated and untreated groups of some significance. The results as given previously indicate quite definitely that as a whole the group that was treated lived a great deal longer than the group that was not treated. It is of interest to note that of the 4 treated animals that died, the death of the last of the 4 occurred 111 days after the animal had received the infective inoculum, whereas 16 (80%) of the 20 animals of the control group that died did so after this time. These data suggest the favorable influence of therapy in prolonging the survival time of the animals in the group that were treated.

*Relative Amounts of Tuberculosis in Treated and Untreated Groups.* Of much interest and importance in determining the effect, if any, of a chemical agent administered with therapeutic intent to tuberculous guinea pigs are the amount and character of the tuberculosis in the treated and untreated groups of animals. With drugs having little, if any, deterrent effect on the disease process sufficient information can usually be obtained by comparing the gross changes in the organs of predilection of the treated and untreated animals. However, in instances in which the gross examination suggests or indicates that the therapeutic agent has exerted a favorable effect on the expected course of the disease, a proper evaluation of the influence of the drug is possible only by a microscopic examination of the tissues. This is especially true in relatively short-term experiments in which there is insufficient time for the disease to express its maximal severity. Microscopic studies will frequently reveal residual disease or relics of previous disease that could not have been detected grossly. One should not be satisfied with less exacting criteria.

Following a scheme which has been described previously,\* a sum-

\* This scheme for evaluating numerically the relative severity of tuberculosis in experimentally infected guinea pigs is based on (1) the premise that a non-progressive or arrested tuberculous process should be assigned a value inferior to that assigned to processes that are progressive and destructive and (2) on the arbitrary selection of the figure 100 as representing the maximal amount of tuberculosis possible in any one animal. A detailed description of the scheme and of its application has been reported previously.<sup>5</sup>

mary of the effects of 4,4'-diaminodiphenylsulfone in the respective experiments is given in Table 1.

TABLE 1.—SUMMARY OF THE EFFECTS OF 4,4'-DIAMINODIPHENYLSULFONE EXPRESSED NUMERICALLY

(All animals were inoculated subcutaneously with H37RV. The dose of the inoculum in Experiments 1 and 2 was 0.1 mg. In Experiment 3, the dose was 0.0005 mg.)

Experiment	Duration (days)	Group	Numerical index of infection determined microscopically				
			Spleen (Max. 35)	Lungs* (Max. 30)	Liver (Max. 25)	Site of inoculation* (Max. 10)	Average index (Max. 100)
1†	60	Control (14 animals)	31.8	16.4	18.2	10.0	76.4
		Treated (13 animals)	1.3	1.7	1.3	7.8	12.1
2†	60	Control (10 animals)	26.1	19.0	16.3	10.0	71.4
		Treated (10 animals)	2.9	3.8	1.2	6.4	14.3
3‡	228	Control (28 animals)	31.3	24.7	18.4	9.7	84.1
		Treated (13 animals)	1.4	2.8	0.23	4.0	8.4

\* Includes contiguous lymph nodes.

† Administration of drug started 2 days prior to infection.

‡ Administration of drug delayed until 42 days after infection.

It may be noted that in the 2 experiments that were terminated 60 days after infection, the average index of infection among the animals that were not treated was 76.4 and 71.4, respectively, whereas for the animals that received the drug the average index of infection was considerably less, being 12.1 and 14.3 respectively. These differences, indicative of a deterrent effect of the drug, become even more impressive if the involvement of the spleen, lungs and liver only is considered and the disease at the site of inoculation is disregarded. With these limitations the figures for Experiment 1 would be: untreated group 66.4,\* treated group 4.3; for Experiment 2 the average numerical index for the untreated group would be 61.4 and for the group that was treated, 7.9.

The differences in the indexes of infection were most striking among the 2 groups of animals utilized in the long-term experiment (No. 3). Among the untreated controls the average numerical index of infection was 84.1 (based on an arbitrary maximum of 100), whereas in those animals that received the drug the average numerical index of infection was only 8.4. If one disregards the involvement at the site of inoculation and the contiguous lymph nodes, the figure for the untreated group is 74.4 compared with 4.4 for the group that was treated.\*

Of considerable significance was the observation that among the 13 guinea pigs treated with 4,4'-diaminodiphenylsulfone in Experiment 3, 6 (46%) were devoid of lesions of tuberculosis—grossly and microscopically—in the liver, spleen and lungs. In contradistinction to the findings just mentioned there was only 1 among the 28 animals that were not treated, in which lesions, although present at the site

\* If the site of inoculation is disregarded the maximal index is 90 instead of 100.

of inoculation and in contiguous lymph nodes, were not demonstrable in the liver, spleen or lungs.

*Pathologic Changes.* Aside from those animals that were treated with 4,4'-diaminodiphenylsulfone and were without lesions of tuberculosis in the organs of predilection (Experiment 3) the most impressive indication that the drug was capable of a tuberculotherapeutic effect was the difference in the histopathologic characteristics of the tissues of the treated and of the untreated animals.

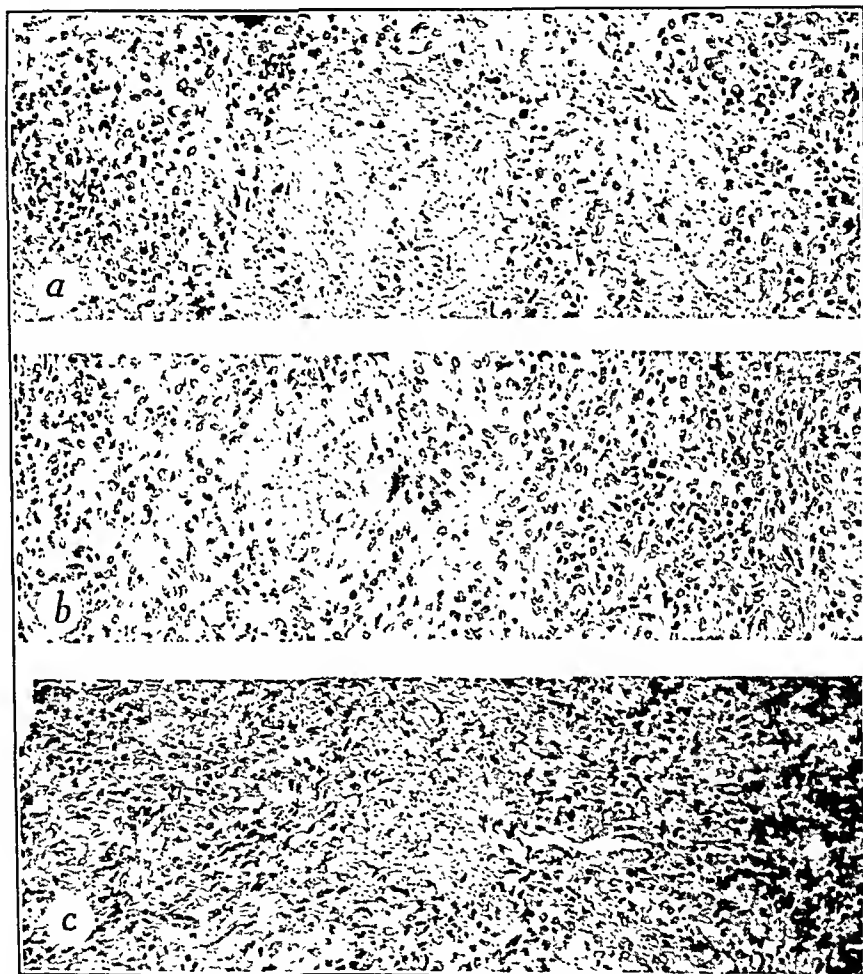


FIG. 3.—Progressive destructive tuberculous changes in (a) spleen, (b) liver and (c) lung of an untreated guinea pig killed 60 days after infection (Experiment 1) ( $\times 150$ ).

In Experiments 1 and 2 the aggressive character of the disease in the untreated controls was in sharp contrast to the physical characters of the disease among the animals that had received the drug. In the former the liver, spleen and lungs were involved moderately or extensively with necrotizing, disseminating and destructive tuberculosis (Fig. 3). When lesions were present in the liver, spleen or lungs of the animals that had received 4,4'-diaminodiphenylsulfone, they were



definitely of a different character from the morbid process that characterized the lesions in the untreated controls. Among the treated

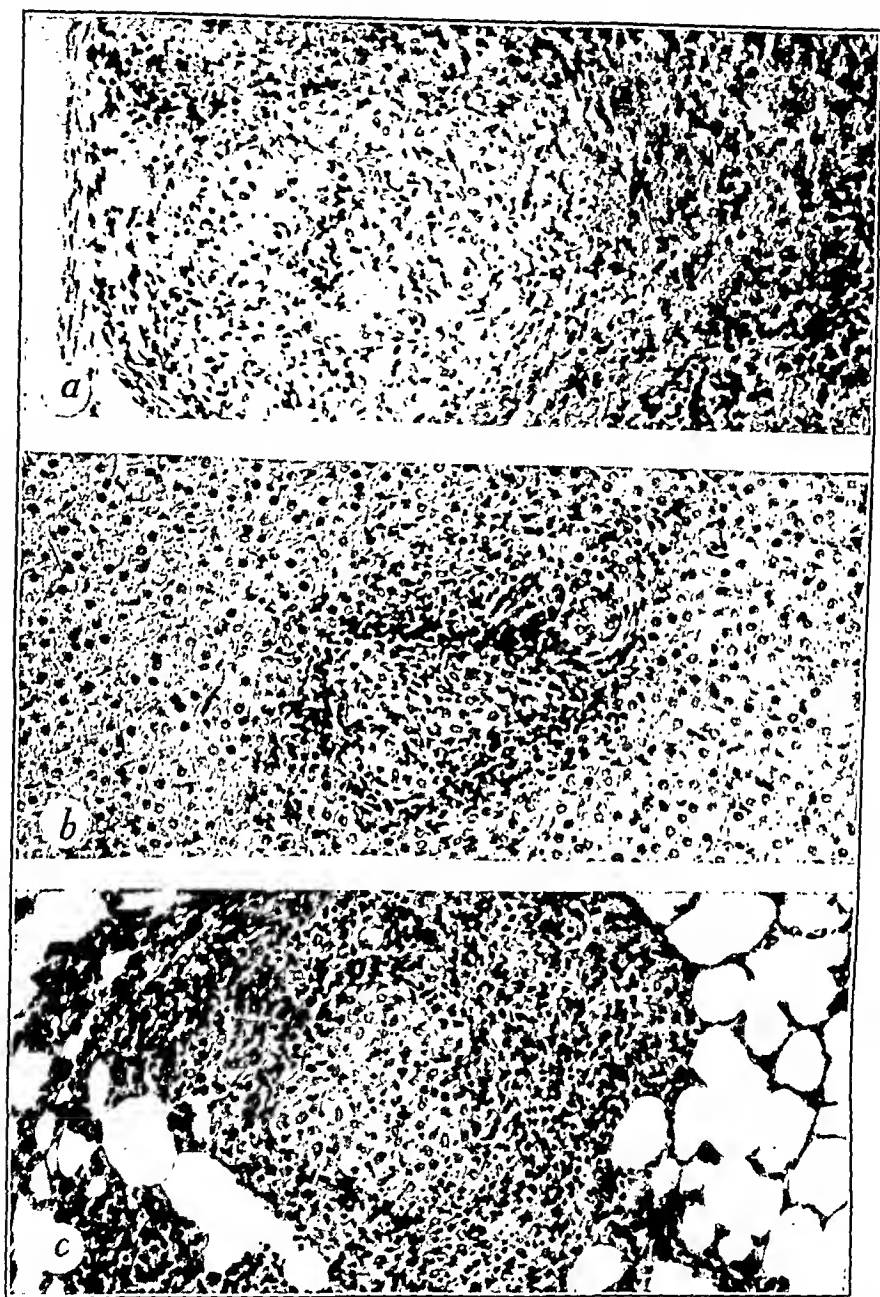


FIG. 4.—Non-progressive tuberculous changes in (a) spleen, (b) liver and (c) lung of a guinea pig that received 4,4'-diaminodiphenylsulfone for 60 days after infection was established (Experiment 1) ( $\times 150$ ).

animals the tuberculous changes of the liver, spleen and lungs were usually focal rather than diffuse and the lesions were relatively few and small. Necrosis was infrequently observed and, when present,

was minimal in amount. The tubercles were often of the so-called hard variety or the epithelioid cells showed retrogressive changes as a

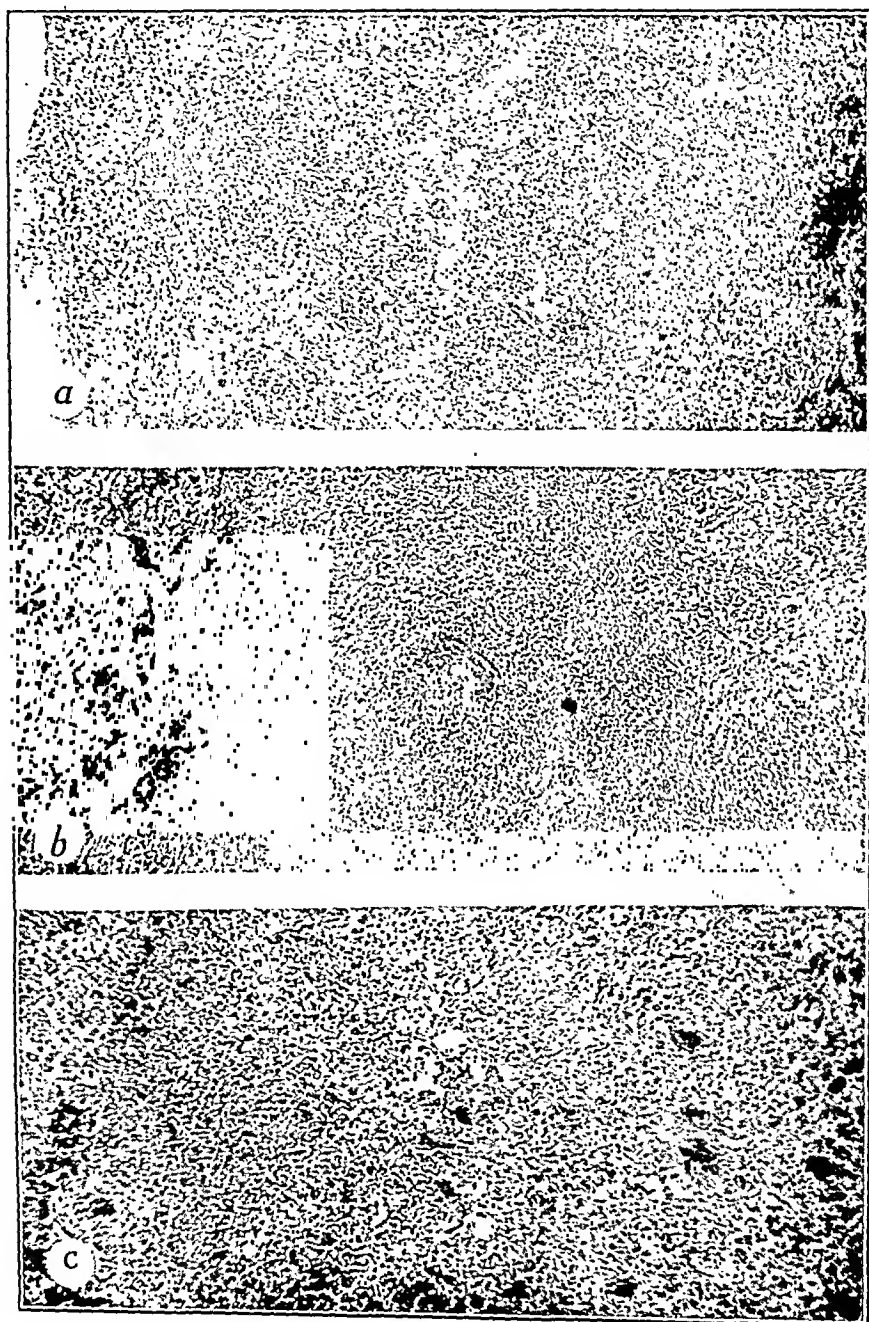


FIG. 5.—Extensive necrotizing tuberculous involvement in (a) spleen, (b) liver and (c) lung of an untreated guinea pig. The animal was killed 228 days after infection (Experiment 3) ( $\times 60$ ).

consequence of which “foam cells” were numerous. A moderate to an intensive accumulation of lymphocytes and histiocytes occurred at the periphery of many of the lesions (Fig. 4).

The pathologic changes that occurred in both groups of animals in Experiment 3 were more dramatic than those of Experiments 1 and 2. The duration of the infection in Experiment 3 (228 days) provided (1) a fuller expression of the pathogenic potential of the infectious agent in the untreated group, and (2) a greater length of time for the drug to exert a favorable influence among the animals that were treated. Parenthetically, it should be recalled that the beginning of treatment in Experiment 3 was delayed until 6 weeks after the animals

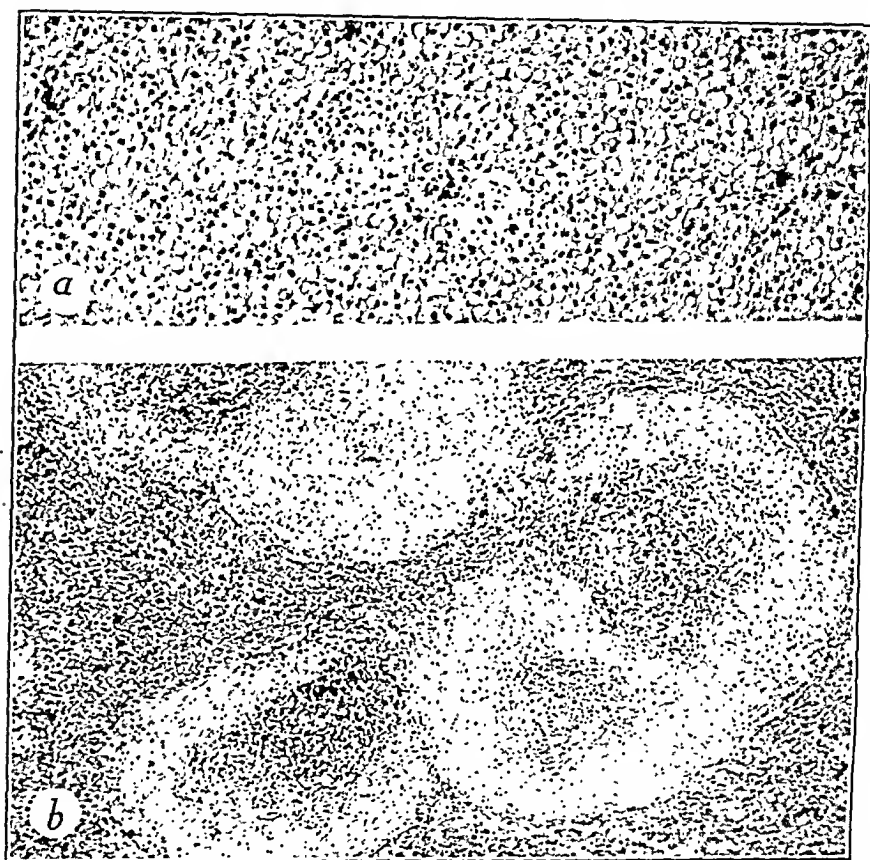


FIG. 6.—(a) Atrophic epithelioid nodules in the liver of a tuberculous guinea pig treated for 69 days with 4,4'-diaminodiphenylsulfone ( $\times 100$ ). (b) Fibroblastic changes of splenic lesions indicative of healing. The animal died after receiving 4,4'-diaminodiphenylsulfone for 34 days ( $\times 75$ ).

had been infected. This fact might be expected to militate against favorable therapeutic effects of the drug.

The histopathologic character of the disease in the untreated animals was that of an apparently uninhibited process that had succeeded in many instances in occupying much, if not most, of the respective organs of predilection. Necrobiosis was a constant and striking feature, and in some instances the parenchymal involvement was so severe as to obliterate identifying histologic "landmarks" (Fig. 5).

Among the animals in Experiment 3 treated with 4,4'-diaminodi-

phenylsulfone for 186 days, as mentioned previously, there were relatively few of the organs of predilection in which residual lesions of tuberculosis could be found. In the one liver in which lesions were seen they consisted of 2 small atrophic epithelioid nodules which were evidently non-progressive, since the animal had been infected 111 days prior to death, having been treated the last 69 days of life (Fig. 6a). The changes observed in the spleen of 1 animal that died after having received treatment for 34 days were of some interest because of the

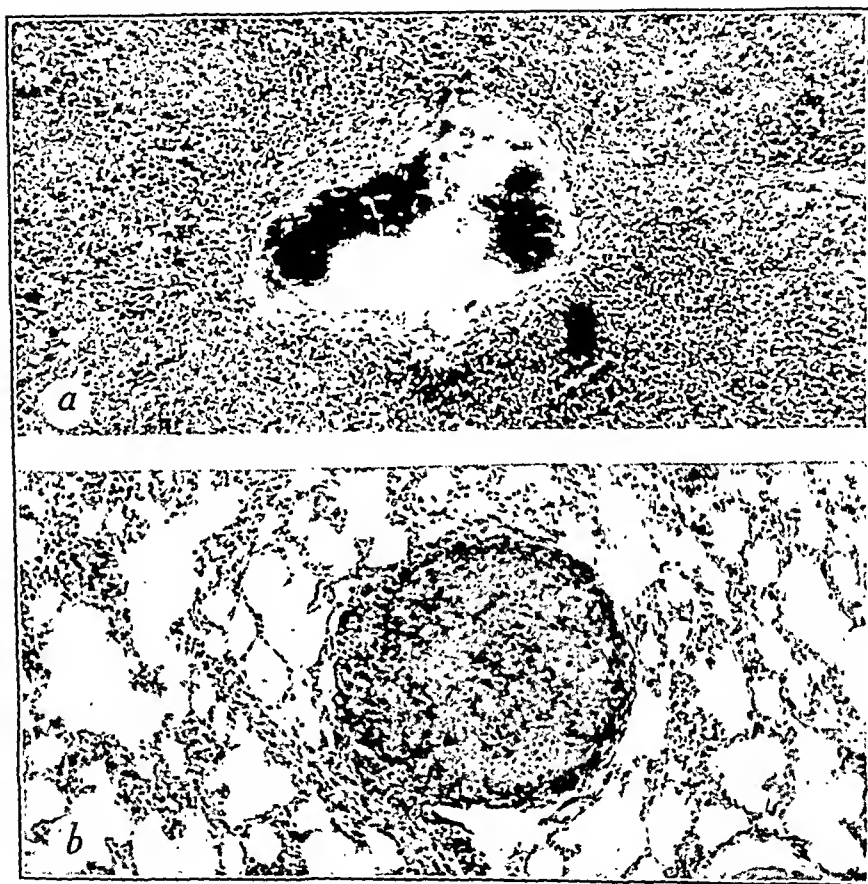


FIG. 7.—(a) Calcified nodule in spleen of a tuberculous guinea pig treated for 186 days with 4,4'-diaminodiphenylsulfone. This was the only lesion found in the organs of predilection in this animal ( $\times 100$ ). (b) Solitary "hard tubercle" in the lung of a guinea pig treated for 186 days with 4,4'-diaminodiphenylsulfone ( $\times 100$ ).

marked fibrosis of what were presumed to be tuberculous changes (Fig. 6b). The 2 spleens that contained lesions among the 10 guinea pigs that were killed when the experiment was terminated showed only a few well-encapsulated calcified nodules (Fig. 7a). The lesions observed in the lungs were few and consisted of "hard tubercles" (Fig. 7b). A finding worthy of mention was an apparent tendency of the lesions of the axillary lymph nodes of the treated animals to calcify.

*Sensitivity to Tuberculin.* Two days before Experiment 3 was terminated the animals that were living were again inoculated with tuberculin intracutaneously. Of the 8 untreated animals tested the results in 6 were definitely positive, in 1 negative and 1 animal died before the test was completed. Each of the 8 animals was extensively tuberculous. Of the 10 guinea pigs that had received 4,4'-diaminodiphenylsulfone for 186 days and which were sensitized to tuberculin when the treatment was started, the results were as follows: 5 definitely positive, 2 indefinitely positive and 3 negative. Of the 5 guinea pigs that gave a definite reaction to tuberculin, lesions of tuberculosis were present in the organs of predilection of only 1. Of the 2 in which the reaction to tuberculin was indefinite, lesions of minimal and non-progressive character were present in the spleen and lung of 1 but the liver, spleen and lungs of the other were free of demonstrable lesions. In 2 of the 3 animals that had negative reactions the parenchymal lesions were limited to non-progressive foci in the lungs, while in the third animal parenchymal lesions were not found.

*Attempts to Culture Tubercle Bacilli From the Spleen.* When Experiments 1 and 2 were terminated, a portion of the spleen of each animal was suspended and used to inoculate culture media suitable for the growth of tubercle bacilli. Of the 14 spleens representing the untreated controls in Experiment 1, tubercle bacilli were cultured from 12; in 2 the results were negative. Of the 13 spleens from the treated animals in Experiment 1, tubercle bacilli were cultured from only 1; in 12 the results were negative. In Experiment 2, of the 10 spleens from the animals that were not treated, positive results were obtained in 9; in 1 the result was negative. Of the 7 spleens cultured from the animals that had been treated, 2 were positive and 5 were negative. When the data from the 2 experiments are summarized it is revealed that from the spleens of 24 untreated animals positive results were obtained in 21 instances, whereas from the spleens of 20 animals that had received 4,4'-diaminodiphenylsulfone, tubercle bacilli were obtained from only 3; the results in 17 were negative.

*Toxicity.* As mentioned previously, 4,4'-diaminodiphenylsulfone has been considered a compound of relatively high toxicity. Our data, although not comprehensive, indicate that guinea pigs weighing 1 pound (0.5 kg.) or more will tolerate at least 150 mg. of the drug daily over a long period when the drug is given in the feed. As with the derivatives of 4,4'-diaminodiphenylsulfone, the parent nucleus does induce certain definite changes in the blood. These changes, while of an adverse character, appear in the guinea pig in the dose we have used not to be critical so far as the life of the animal is concerned. The changes are reversible and when the administration of the drug is discontinued the blood is restored to a normal state within a relatively short time. Furthermore, in the dose used, the animals ate well and maintained a presumably normal rate of growth and weight.

*Changes of the Blood.* Although no attempt was made to obtain detailed information concerning the effect on the blood of 4,4'-diaminodiphenylsulfone in the 3 experiments described, some data were secured

through the kindness of our colleague, Dr. George M. Higgins. At the termination of Experiment 3 (228 days after infection) blood was obtained by cardiac puncture from the 8 surviving animals in the control group and from 6 of the animals that had been receiving 4,4'-diaminodiphenylsulfone for 186 days. The results of the study of the blood are summarized in Table 2.

TABLE 2.—EFFECTS OF 4,4'-DIAMINODIPHENYLSULFONE ON THE BLOOD OF TUBERCULOUS GUINEA PIGS

(The treated animals had received the drug daily in the feed for 186 days)

	Animals	Erythrocytes (millions per c.mm.)	Volume of erythrocytes (cu. $\mu$ )	Hemoglobin (gm. per 100 cc. of blood)	Reticulocytes (% of erythrocytes)
Control	8	5.35 $\pm$ 0.05*	84.9 $\pm$ 0.90	13.3 $\pm$ 0.15	1.3 $\pm$ 0.17
4,4'-diaminodiphenylsulfone	6	3.63 $\pm$ 0.14	123.1 $\pm$ 2.30	11.2 $\pm$ 0.50	10.3 $\pm$ 0.80

\* Probable error of the mean.

From the data assembled, it appears that definite destruction of the erythrocytes was exerted by the drug. A hypochromic macrocytic type of anemia was induced. The total number of erythrocytes per c.mm. of blood from the treated animals was 1,720,000  $\pm$  150,000 less than the controls, and the average size of the erythrocytes from the treated animals was 38.2  $\pm$  2.5 cu.  $\mu$  greater than the controls. This degree of macrocytosis indicates rapid regeneration by the hemopoietic centers. The concentration of hemoglobin per 100 cc. of blood in animals given the drug was 2.1  $\pm$  0.5 gm. less than the controls, a difference which is statistically significant. The high percentage of reticulocytes (10.3  $\pm$  0.8) is further evidence that the hemopoietic centers were not impaired by the drug, which apparently acts directly on the erythrocytes in the circulation.

The concentrations of hemoglobin and of 4,4'-diaminodiphenylsulfone in the blood of the animals in Experiments 1 and 2 are shown in Table 3.

TABLE 3.—CONCENTRATIONS OF HEMOGLOBIN AND OF 4,4'-DIAMINODIPHENYLSULFONE IN THE BLOOD OF GUINEA PIGS THAT HAD RECEIVED THE DRUG FOR 60 DAYS

Experiment	Group	Animals	Average grams of hemoglobin per 100 cc. blood	Average concentration of 4,4'-diaminodiphenylsulfone per 100 cc. of blood
1	Control	14	13.2 (11.2–15.5)*	1.94 (1.41–3.45)
	Treated	13	11.3 (9.1–12.8)	
2	Control	10	13.3 (11.2–15.7)	4.1 (1.88–5.76)
	Treated	7	10.1 (1.9†–12.4)	

\* Figures in parentheses represent ranges.

† The guinea pig with the hemoglobin value of 1.9 gm. had, when killed, severe hemorrhagic colitis and a few petechial hemorrhages in the mucosa of the stomach. The lesions mentioned could have accounted for the low hemoglobin value, as a result of hemorrhage.

**Comment.** These studies, while not exhaustive, do provide sufficient data to establish the fact that 4,4'-diaminodiphenylsulfone under the conditions imposed has a relatively high tuberculotherapeutic efficacy. Not only was the drug capable of exerting a marked deterrent effect on the development of tuberculosis in guinea pigs when the administration of the drug was begun 2 days before the animals were infected, but of much greater importance was the favorable effect achieved when the beginning of treatment was delayed until 6 weeks after the animals

had been inoculated. Conservatively, one must consider the data obtained from the first 2 experiments, which were terminated 60 days after infection, as suggestive rather than convincing evidence of the favorable effect of the drug. The results of the 3rd experiment, which continued for 228 days, were entirely consistent with the situation that appeared so impressive in the experiments of shorter duration. In other words, even though the infective dose of tubercle used in Experiments 1 and 2 was 200 times as great as that used in Experiment 3 and the period of treatment was only 60 days compared with 186 days in Experiment 3, a definite and anatomically significant effect of the drug was demonstrable. In the experiment of longer duration the same favorable anatomic picture was present and the therapeutic effects were more apparent. Fewer lesions were found, and those observed showed a more complete arrest of the disease than occurred in the animals that received the drug for 60 days only.

Whether or not treatment for a period longer than 186 days would have reduced the residual evidence of tuberculous infection still further is problematic, but it would appear to be a reasonable presumption that the retrograde processes evident when Experiment 3 was terminated would have proceeded if the administration of the drug had been continued for an indefinite period.

The data, while suggestive that the drug had exerted a deleterious influence on the tubercle bacilli, are hardly sufficient to justify the conclusion that no viable tubercle bacilli remained in the tissues even in instances in which no lesions were found and in which splenic cultures were negative. To determine this point with certainty, it would be necessary to continue the experiment indefinitely after the period of treatment so as to permit the unhampered development of possible latent infections. However, the facts do indicate that 4,4'-diaminodiphenylsulfone, like several of its derivatives, is capable, in the highly susceptible guinea pig, of coping successfully with a progressive tuberculous process already well established when treatment is started. Even if one assumes that not all the tubercle bacilli are rendered non-viable or destroyed, the treated animal in most instances is able, as a consequence of the drug, to bring to bear on the infective process an enhanced mechanism of resistance that succeeds at least in arresting the disease. This is obviously a highly desirable effect.

The ideal tuberculotherapeutic drug should destroy or inactivate all tubercle bacilli, but it is doubtful if such a goal need be attained. While such a result might be necessary actually to "cure" the disease in the guinea pig, it is generally agreed that this is not necessary in human tuberculosis. Even such successful therapeutic measures as collapse therapy merely render aid to the natural defenses of the human host, without directly or necessarily contributing to the extermination of the tubercle bacilli. It does not seem logical that one should insist that a drug possess powerful bactericidal properties before one considers it for human administration, if bacteriostatic properties would suffice to attain the objectives of treatment.\*

\* Some pertinent considerations of clinical tuberculochemotherapy have been discussed previously.<sup>6</sup>



**Summary and Conclusions.** To determine the effect of 4,4'-diaminodiphenylsulfone on the expected course of experimental tuberculosis in guinea pigs, 3 experiments were done. In 2 of the experiments the administration of the drug was started 2 days before the infective agent was introduced and the experiments terminated 60 days after infection. In the 3rd experiment the animals were inoculated with tubercle bacilli 6 weeks before treatment was started. The 3rd experiment was terminated 228 days after infection. All animals were inoculated with the virulent variant of H37 (H37RV). The daily dose of the drug was approximately 150 mg. incorporated with the feed. In the dose employed, the drug exerted only a moderate toxic effect on the blood. Favorable effects of the drug on the infection were definitely apparent in the 2 experiments that were terminated 60 days after infection. These effects were more complete and convincing in the experiment that continued for 228 days than in the short-term experiments. In the long-term experiment, although 71.4% of the untreated animals had died, in the treated group only 28.6% of the animals had died at the conclusion of the experiment. Great differences occurred also in the character and amount of tuberculosis in the treated and untreated groups of animals, those that received the drug either being without demonstrable lesions or having residual foci in which the process was arrested, calcified or healed.

Under the conditions of the experiments, the results indicate (1) that 4,4'-diaminodiphenylsulfone is an effective agent in inhibiting or in combating experimental tuberculosis of guinea pigs; (2) that continuous prolonged administration yields cumulative benefits not attained in short-term experiments; (3) that the drug is not excessively toxic for guinea pigs in the dose used; and (4) that efforts to modify 4,4'-diaminodiphenylsulfone to obtain a compound more suitable for clinical application should be encouraged, since the possibilities seem many.

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## EXPERIMENTAL HUMAN INFLUENZA\*

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THE experimental production of influenza in human volunteers has great value in testing methods of prevention and treatment which show promise in the laboratory. Relatively small groups of healthy volunteers, well controlled, offer unusual opportunities for the evaluation of new prophylactic and therapeutic methods. Smorodintseff<sup>20</sup> *et al.* inoculated 72 volunteers by inhalation with a 10% suspension of mouse lungs infected with the Leningrad or the W.S. strain of virus. Each volunteer inhaled a calculated  $10^5$  to  $10^6$  minimum lethal mouse doses of virus during exposures of from 15 to 60 minutes. Twenty per cent of inoculated individuals showed symptoms diagnostic of influenza. A definite correlation was found between the initial protective titer of the blood and susceptibility to experimental infection, all cases showing clinical symptoms having low initial titers. Increases in neutralizing titers after exposure were noted in most of the volunteers, especially in those showing clinical symptoms following the inhalations.

Burnet and Lush<sup>4</sup> inoculated approximately 150 individuals by intranasal spraying of an egg passage strain of Melbourne virus avirulent for ferrets and mice. No symptoms were observed in exposed subjects and little or no antibody production was demonstrable in a sample group tested.

Chalkina,<sup>6</sup> in experiments on the immunization of volunteers by inhalation of active virus, sprayed a total of 272 men with varying concentrations and doses of mouse lung virus. Definite antibody responses were observed following such exposure and, by controlling the quantity of virus inhaled, undesirable symptoms could be prevented. Of a group subjected to the highest concentration for 30 to 45 minutes, 20% developed symptoms diagnostic of influenza.

Burnet and Foley<sup>5</sup> subjected 15 individuals to repeated insufflations of several viruses, beginning with an avirulent egg-adapted Melbourne strain and concluding with a recently isolated virus. Three of the subjects, all of whom had low initial titers, developed definite symptoms of influenza. The authors emphasize the correlation between low initial antibody level and susceptibility to experimental infection.

Francis<sup>7</sup> inoculated 11 human subjects by intranasal instillation with approximately 500 minimum lethal mouse doses of virus. Only

\* The opinions advanced in this publication are those of the writers and do not represent the official views of the Navy Department.

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1 of the subjects showed a definite antibody rise subsequent to the inoculation while none exhibited significant symptoms of influenza.

Henle, Henle and Stokes,<sup>10</sup> employing a strain of virus (F-99) recently isolated from a fatal case, were able to produce influenzal symptoms with temperatures over 100° F. in 10 out of 28 control subjects, and in 1 out of 27 vaccinated subjects. Serologic tests confirmed the observation of Burnet and Foley on the relationship between initial antibody titer and susceptibility to the experimental infection. It was pointed out that individuals with high initial titers subjected to inhalation of the virus did not respond with a significant increase in antibody as did many of the high titer subjects who were vaccinated intramuscularly.

It was our original intention to study the efficacy of immune serum administered by inhalation in the prophylaxis and treatment of experimental human influenza. The whole-hearted coöperation of the officials and several hundred inmates of a California state penal institution was obtained and the preliminary work on optimum infectious dose begun. The project was terminated in this early stage (March, 1943) on orders from the Navy Department, Bur. of Med. and Surg. However, the preliminary data obtained were considered to be of some interest and it is the purpose of this paper to present the methods used and results obtained.

**Methods. Examination of Volunteers.** All volunteers for the experiment were healthy adult males who were subjected to a thorough allergy history questionnaire, skin and conjunctival tests to determine sensitivity to normal horse serum, and a physical examination of the nose, throat, and chest. The questionnaire and allergy tests were conducted in anticipation of the use of the influenzal immune horse serum concentrate. Blood samples were taken by venepuncture at the time of examination, and after clotting the serum was separated by centrifugation, inactivated at 56° C. for 30 minutes, and stored at 4° C. until tested.

**Serology.** Pre- and postinhalation (2 week interval) serum titers were determined by a red blood cell agglutination inhibition test<sup>15</sup> utilizing human red cells with a constant (1:100) dilution of serum titrated against increasing dilutions of agglutinating virus. Titers are expressed in terms of the fold difference in end-point of agglutination between the test sample and a normal rabbit serum (1:100 dilution) control series. For example, if a test serum has its end-point of agglutination in the tube containing a 1:80 dilution of virus and the normal rabbit serum titer of the virus is 1:2560, the fold difference, or titer, is regarded as 32.

**Spraying of the Virus.** For the purpose of administering active virus by inhalation we employed glass atomizers of the type described in a separate publication.<sup>16</sup> One of these atomizers was connected at each end of a metal cylinder, 5 feet long and 7 inches in diameter, with 3 openings on each side to which rubber anesthetic masks were attached with flexible rubber tubing (Fig. 1). The outlet valve of the masks allowed escape of some virus fog into the air of the room, but the dilution factor of the air and other precautions taken (see below) minimized the chances of infection of attending personnel. Air at a pressure of 40 cm. Hg was supplied to the atomizers from an electrically driven air compressor. Equilibrium conditions for virus concentration within the chamber were obtained by preliminary atomization for 5 minutes, during which time one outlet functioned through cotton soaked in 5% lysol.

By previous calibration of the atomizers<sup>16</sup> as to air and fluid delivery it was possible to compute the concentration of virus in the air inspired by the

men. Upon completion of the preliminary period of atomization, the air pressure was discontinued momentarily and the connecting tubes immediately attached to the masks on the test subjects. The pressure was again raised to 40 cm. Hg and held until the period of exposure was completed. After removal of the masks the subjects were then hospitalized for at least 5 days either in a small isolated ward room containing 6 beds or in individual isolated cubicles containing 1 bed. The 1 door to the ward was protected by a curtain of ultraviolet light and the room was maintained in a constant atmosphere of propylene glycol dispensed by immersion of an electric light bulb in the solution.<sup>17</sup> The concentration was held at approximately 0.1 mg. propylene glycol per 1000 cc. of air. Similar precautions were taken in the corridor adjacent to the individual rooms housing test subjects and in the room in which the virus spraying was conducted. The insides of the masks were swabbed with 70% alcohol between test groups.

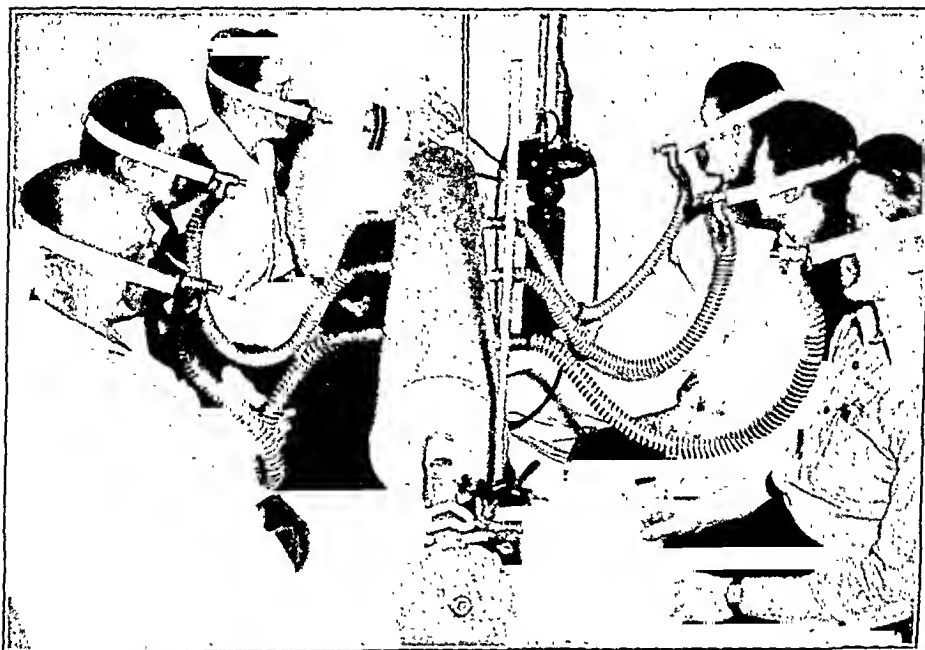


FIG. 1.—Method of administration of influenza virus to volunteers.

**Experimental.** The preliminary publication by Stokes and Henle<sup>21</sup> on the successful mild infection of humans with a recently isolated "A" type strain of influenza virus (F-99) suggested the possible use of this same infectious agent for our experimental purposes. Dr. Stokes kindly supplied this laboratory with an F-99 infected mouse lung in glycerin. This virus was passed through 4 series of eggs by allantoic inoculation and the final product obtained gave a 50% mortality end-point of  $1 \times 10^{-6.6}$  and a red blood cell agglutination end-point of 1:640. This virus suspension, after testing for bacterial sterility, was stored in sterile lüstéroid tubes in the dry-ice chamber until ready for use, at which time it was thawed in warm water until clear and then atomized into the chamber for the period scheduled. It was calculated that if the average subject breathed at the rate of 12 liters per minute, approximately 0.3 cc. of the virus was inhaled per minute.

The proportion exhaled is an unknown, although it has been estimated by Heubner<sup>11</sup> to be between 50 and 80% of the quantity inhaled.

Six groups of 6 men each were successively sprayed with this fluid, the exposure periods being increased on each succeeding group until 1-, 3-, 6- and 12-minute exposures had been attempted. No significant symptoms were observed in any of the 24 test subjects. Blood counts showed no significant changes. Pre- and postinhalation titers against F-99 egg agglutination virus were determined by the red blood cell agglutination inhibition method. Only 4 individuals out of the 24 showed a four-fold or greater antibody titer rise.

During the period of investigation of the infectivity of the F-99 virus, a number of cases of suspected influenza appeared in a Naval training station in this area. Rhinopharyngeal washings were obtained from 5 patients exhibiting symptoms of influenza.<sup>18</sup> In each case 20 to 25 cc. of nutrient broth, pH 7.1, were injected into the rhinopharynx through a rubber catheter. The subject was instructed not to swallow the broth but to eject it through the mouth into an emesis basin. The washings were then transferred to a sterile lusteroid tube and immediately stored in a dry-ice chamber.

Various methods for the isolation of influenza virus from such material have undergone trial in numerous research laboratories since the original isolation of the virus in the ferret by Smith, Andrewes and Laidlaw.<sup>19</sup> While the ferret has proved of great value in the study of influenza epidemics,<sup>14</sup> mice, tissue culture media, and the chorio-allantoic membrane of the chick embryo also have been advocated for primary virus isolation.<sup>8,9,10</sup> Taylor and his associates have reported that hamsters compare favorably with ferrets for direct isolation from nasopharyngeal washings.<sup>22,24,25</sup> Burnet<sup>2,3</sup> has successfully applied the amniotic inoculation of chick embryos, pioneered by Buddingh and Polk<sup>1</sup> in their study of meningococcal infections. He claims that unadapted influenzal virus is capable not only of infecting the chick embryo, but of producing easily recognizable effects in the embryo as well. The use of chick embryos for rapid isolation and identification of virus received a great stimulus from the work of Hirst<sup>12,13</sup> on the hemagglutinability of virus-containing allantoic fluid. The application of this phenomenon has measurably reduced the time required for isolation and identification of influenza strains.

The procedure employed on the 5 rhinopharyngeal washings obtained from Naval personnel was as follows: An attempt was first made to break up the clumps of mucous material in the thawed sample by repeatedly filling and emptying the washings through a 30 cc. syringe fitted with a 20 gauge or 23 gauge needle. The sample was then angle-centrifuged at 5000 r.p.m. for 20 minutes and subsequently 0.1 to 0.2 cc. of the supernatant fluid was inoculated into the amniotic sac of each of eight 13-day old embryos, according to the technique described by Taylor and Chialvo.<sup>23</sup> Following 36 to 40 hours incubation at 37° C. blood-free allantoic fluid from each of the live embryos, and a few dead embryos, was collected and a 10% saline suspension of the pooled, ground tracheas was prepared. Alundum and the larger tissue

partieles of the tracheal suspension were removed by low-speed centrifugation. The allantoic fluid from each embryo and the tracheal suspension were then tested for the presenee of hemagglutinins. If these proved negative, 0.1 to 0.2 cc. of the tracheal fluid was re inoculated into the amniotic sac of each of 8 embryos and the described procedure repeated.

Of the 5 rhinopharyngeal washings tested, 2 showed the presence of virus in the first egg passage, and the remaining 3 were positive by the third egg passage. Inhibition tests of each virus against PR8 and Lee rabbit-immune sera indicated all 5 to be "A" type. There appeared definite variation in the adaptability of the viruses for mice, 2 of the strains requiring but 1 egg passage to become lethal for mice while the remaining 3 required up to 7 passages to attain this degree of virulence.

Attempts to isolate virus from these same washings by means of hamsters, according to the technique of Taylor,<sup>25</sup> or by direct inoculation into mice, proved entirely negative.

One of the viruses appearing in the first egg passage was inoculated into a group of mice, from which 2 further egg passages into the allantoic fluid of 11 day old embryos were made. This final egg passage furnished the virus used for infection of the next series of volunteers. It had an agglutination end-point of 1:1280, which was inhibited by a PR8 immune rabbit serum, but not by a Lee immune serum. The same technique of human inoculation was employed with this virus and 3 exposure periods were tested as follows: 6 men for 6 minutes, 5 men for 10 minutes, and 6 men for 12 minutes. These subjects were likewise hospitalized and blood counts performed on the 1st, 3rd and 5th day in some and every day in others by inmate personnel. Temperature readings were taken at least twice a day.

Of these 17 volunteers, 10 showed signs and symptoms of influenza. Of the 10, 4 were regarded as typical clinical influenza and 6 as highly suggestive of this disease. Table 1 summarizes the occurrence of the various signs and symptoms in these 10 cases.

TABLE 1.—SUMMARY OF OCCURRENCE OF VARIOUS SIGNS AND SYMPTOMS IN 10 VOLUNTEERS SHOWING SIGNS OF INFLUENZA

Symptom	No. of volunteers showing symptom
Fever over 100° F.	7
Feeling of feverishness	10
Malaise	10
Headache	9
Backache	6
Generalized ache	5
Tightness in chest	2

The 3 men who reported feeling feverish, but who are not included in those showing a temperature over 100° F., very likely would have shown a significant temperature had the readings been taken at the proper time. It was believed that the peaks had been missed in these cases, although the transitory nature of the temperature rises indicated only a mild reaction.

The onset of the fever and symptoms occurred 9 to 12 hours following exposure in 3 of the subjects, and between 24 and 30 hours in the remaining 7. No signs of upper respiratory infection were observed in any of the patients, nor were any indications of lung involvement noted. The blood counts showed no significant changes, but there is reason to believe that the blood counts undertaken by inmate personnel were not entirely reliable. Henle, Henle and Stokes<sup>10</sup> noted a significant leukopenia (below 5000) and a relative lymphocytosis in a considerable proportion of their subjects, in those showing no outward signs of infection as well as in the outright cases. It was our intention to follow this aspect of the experiment more closely and perform the counts ourselves had it been possible to continue our experiments. We had the distinct impression that the leukopenia accompanying naturally occurring influenza was an inconstant finding and of doubtful value in diagnosis.<sup>16</sup>

A summary report of 1 of the 4 definite cases resulting from the inhalation of the virus follows:

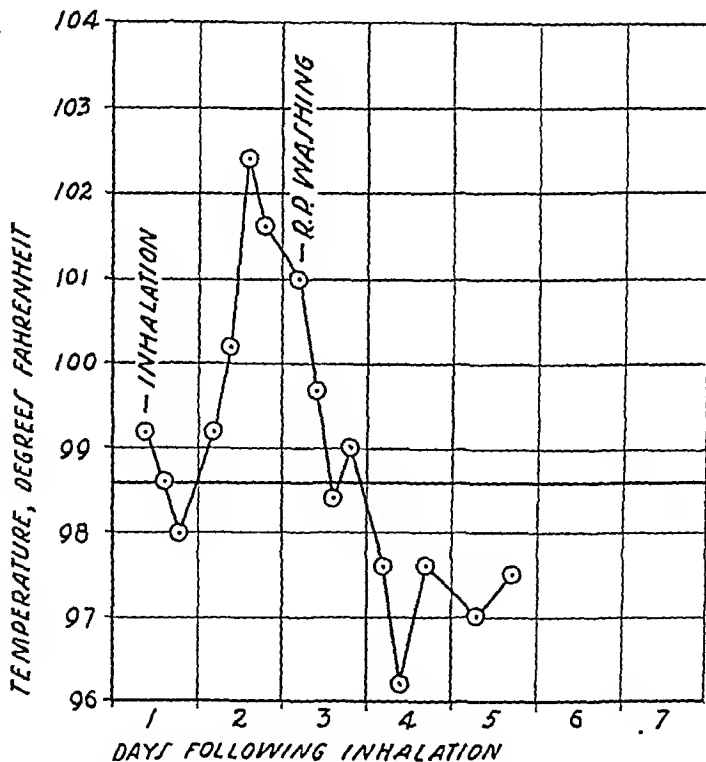


FIG. 2.—Temperature chart on "R. C." exposed to influenza virus 6 minutes.

**Case Studies.** "R. C."—preliminary physical examination was negative. Subject was exposed 3/9/43 to a spray of TI 2A influenza virus for 10 minutes and then hospitalized. About 9 hours after the inhalation period he felt feverish and developed general malaise. This was followed shortly by headache, backache, aching legs, and a sense of "tightness and burning" in the chest. The temperature rose to 102.4° F. and remained elevated about 36 hours. Upon physical examination the facies appeared flushed and dry with mild con-

junctional injection. The lungs were clear. The spine was moderately stiff. The aches and pains, malaise and headache persisted for 48 hours and were followed by general weakness for 48 hours. Daily blood counts showed no significant changes. The temperature chart on this case is presented in Figure 2.

Approximately 48 hours after inhalation of the virus, rhinopharyngeal washings were obtained from this subject and the technique for the isolation of virus followed as described. By the 6th egg passage a positive agglutinating virus appeared which was inhibited by a PR8 immune rabbit serum while unaffected by a Lee immune serum.

Results of the agglutination inhibition tests on all 17 men against the specific TI virus are presented in Table 2.

TABLE 2.—RESULTS OF AGGLUTINATION INHIBITION TESTS

Subject	Time of exposure (min.)	Symptoms	Preinhalation titer	Postinhalation titer	Fold rise in titer
E. S.	6	Suggestive	4	8	2
E. F.	6	Suggestive	0	8	8
W. S.	6	Suggestive	0	8	8
N. W.	6	Suggestive	4	8	2
W. F.	6	Suggestive	16	16	0
J. C.	6	Definite	0	128	128
R. C.	10	Definite	0	32	32
J. L.	10	Definite	0	4	4
E. A. G.	10	None	8	32	4
C. E.	10	None	8	64	8
E. H.	10	None	2	8	4
R. D.	12	Definite	0	2	2
F. G.	12	Suggestive	0	64	64
N. M.	12	None	0	8	8
L. T.	12	None	0	32	32
E. G.	12	None	0	4	4
G. B.	12	None	8	32	4

All 4 subjects who were classed as definite cases showed no antibody titer in the initial serum sample as tested by the method used in this laboratory. Two of these (J. C. and R. C.) exhibited strong antibody response following the inhalation of virus, while the other 2 (R. D. and J. L.) responded with only two- and four-fold rises respectively. The remaining test subjects did not show a definite correlation between the preinhalation antibody level and susceptibility to the experimental infection. The one showing the highest initial titer in the group (W. F.) was classed as suggestive and did not respond with any increase in antibody. On the average, the increases in antibody titers were greater in those individuals showing no original titer.

**Discussion.** The successful isolation of the 5 strains of virus from as many rhinopharyngeal washings by the use of the egg technique indicates to us the value of this medium. When combined with the hemagglutination phenomenon it presents a rapid and simple method of virus identification. In some cases bacterial contaminants will present difficulties necessitating the use of a filter, which no doubt removes considerable virus as well as bacteria, but at present no other certain method is available. Hirst<sup>13</sup> has recently discussed these points.

The failure to produce clinically recognizable infection with the F-99 strain of virus, even with doses several times that employed by Henle,

Henle and Stokes, may possibly be due to the extra egg passages through which this virus was carried before inoculation of our test subjects. The mouse lethal titer of the suspension used compared favorably with the titer reported in their successful experiment, but, of course, such measurements are no guarantee of infectiousness for humans. It is not easily understandable, however, why a virus infective for man after having been passed 16 times in experimental animals and 3 times in eggs should, after but 4 more egg passages, be so innocuous. It may be that virulence alterations of influenza viruses take place in sudden steps rather than by gradual decline. If such is actually the case, a few passages from human to human under proper conditions might bring about the reverse transition, thereby fulfilling the primary requirement for development of a severe epidemic. The possibility of such an occurrence makes one hesitate before advocating immunization of humans by means of attenuated virus administered by inhalation.

Clinical experiments on infection of human volunteers with influenza virus, when precautions of isolation are taken, do not seem to constitute a hazard either to the test subjects or to other individuals in the vicinity. The usual report from human experiments thus far performed is: "No complications encountered."

Our results with the inoculation of 17 men with a recently isolated "A" type virus, TI 2A, would seem to emphasize the tremendous individual variation encountered, not necessarily related to blood antibody level. Among the 10 subjects who showed no preinhalation titer in our test group, the inhalation of virus caused definite symptoms in 4, suggestive symptoms in 3, and no clinical reaction at all in the remaining 3. Only 1 of the total of 17 did not respond with any antibody rise and, significantly, he had the highest initial titer.

Questions concerning immunity to influenza seem to be answered only partially by data on neutralizing content of the blood or nasal secretions. The part played by the tissue involved in the virus-host reaction is as yet vague and difficult to assess.

**Summary.** Twenty-four human volunteers exposed to a fine spray of F-99 egg virus from 1 to 12 minutes showed no clinical symptoms of influenza.

Five "A" type strains of virus were isolated from 5 suspected cases of influenza by means of direct egg inoculation. Methods using hamsters and mice for direct isolation proved negative on the same samples.

One of these strains, TI 2A, was used for infection of a second series of volunteers in preliminary work on optimum infectious dose. Of 17 subjects exposed from 6 to 12 minutes, 4 exhibited definite symptoms of influenza, 6 were highly suggestive, while the remainder showed no significant reactions. The blood counts, as reported to us, revealed no changes. No complications were encountered.

The 4 definite cases all had low initial antibody titers. All but 1 of the 17 test subjects showed some antibody rise as a result of the inhalation. The exception had the highest initial antibody titer of the group.



An "A" type virus was isolated from one of the definite cases. No other attempts at isolation were made in this group.

We acknowledge with pleasure the helpful coöperation of Mr. Clinton T. Duffy, Warden of San Quentin Prison, Dr. Alex Miller, Chief Surgeon, and the members of the California State Prison Board. We also wish to express appreciation for the technical assistance rendered by Mr. James W. Ford.

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#### A NOTE RECOMMENDING THE USE OF DRIED PLASMA OBTAINED FROM FRESH CADAVER BLOOD\*

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BECAUSE of the urgent and immediate wartime need of large amounts of plasma, it was suggested in a previous paper,<sup>1</sup> and indirectly in another,<sup>4</sup> that cadaver blood be processed into dried plasma or, preferably, into dried albumin (since albumin is the effective anti-shock

\* As the chief objection to the use of suitably collected postmortem blood seems to be one of sentiment, we are glad to publish this note recommending the procedure. Especially today, when so much effort and expenditure of valuable working hours are being put into Blood Donation, it would seem that this method, granted of course that the product was sterile and innocuous, might well prove to be a desirable auxiliary or substitute.—EDITOR.

fraction and is precipitated from plasma by the aid of alcohol-water mixtures), because it is available in large quantities at the undertaking establishments of the United States and, of course, can be obtained without initial expense.<sup>2</sup> Unfortunately, the use of cadaver blood is looked upon with some disrepute in this country;<sup>1</sup> there is "something revolting to Anglo-Saxon susceptibilities in the proposal of using cadaver blood,"<sup>3</sup> but this feeling has been overcome in other countries such as Russia.<sup>5-8</sup> It can be pointed out that no one objects to receiving products of dead animals, such as liver extract, insulin, gelatin, isinglass (fish bladders), hormones, enzymes, and so forth, or to the transplantation of corneæ, nerves and, I am told, even of skin of cadavers; and that the blood of cadavers can, if transfused, "live on" and, therefore, would not be subjected to decomposition with the cadaver. In spite of the existence of this stigma, permission was obtained from the hospital authorities to administer the plasma of a recently deceased cadaver to a patient with a malignant disease. The dried plasma was restored to one-fourth its original fluid volume, injected, and the changes observed. Such a procedure apparently has not been recorded before.

**Technique.** Through the coöperation of Oliver H. Bair Company, a Philadelphia undertaker a cadaver was obtained. The individual had died suddenly of "heart disease" some 8 hours previous to the withdrawal of blood. The usual undertakers' type of artery and vein cannulæ were thoroughly washed, sterilized and aseptically inserted into the brachial artery and vein, as requested by the undertakers. (Yudin<sup>7</sup> has shown that the easiest way to obtain blood from a cadaver is to cut the jugular vein in two and to insert a glass U-shaped cannula into the vein above the cut and a long glass cannula, which reaches to the right auricle, into the vein below the cut. The cadaver is placed in the Trendelenburg position, and the blood flows out by gravity. Blood is collected from the systemic veins thereby and not from the portal system, the blood from which could be contaminated easily with bacteria from the gastro-intestinal tract. Yudin collects from 1.5 to occasionally as much as 4 liters of blood per cadaver by this method. He recommends that the individual be dead no longer than 8 hours.) Five pints of sterile saline was forced into the arterial system with a sterilized rubber bulb pump, and 4 pints of cadaver blood mixed with saline were obtained by the "closed" system. The vein cannula was connected by rubber tubing to a sterile needle which was inserted into transfusion bottles (Hospital Liquids Incorporated) in which a partial vacuum exists. The 4 bottles were centrifuged. The 1st and 3rd bottles containing sodium citrate had total volumes of 510 and 575 cc. and 160 and 70 cc. of packed red blood cells respectively; the 2nd and 4th bottles, which contained no citrate (evidences of clotting were noted 4 to 6 hours after withdrawal), had total volumes of 500 and 600 cc. and 40 and 25 cc. of packed red blood cells respectively. The plasma of the 1st and 3rd bottles was combined, cultured, frozen in glass ampoules, dried by the Adtevac process<sup>1</sup> and recultured after being dried; the serum of the 2nd and 4th bottles was treated likewise: 30.5 gm. of dried plasma and 14 gm. of dried serum were obtained.

The plasma and serum were restored to one-fourth of their original volumes with distilled water<sup>1</sup> and administered intravenously to a patient with a teratoma (but who was in "good" physical health). Both plasma and serum were given after a lapse of more than 10 days to note if antibodies might have developed. The patient finally succumbed on December 1, some 6 or 7 weeks after the last administration of cadaver plasma. The microscopic examination of the organs revealed no changes that might be ascribed to the use of cadaver plasma.

**Results.** The serologic and cultural studies were negative. As can be observed in Table 1, the dried cadaver plasma and serum seemed to have similar effects as did the dried donor plasma. No reactions, febrile, anaphylactoid, urticarial, hemolytic or antigenic, were noted in the patient.

TABLE 1.—THE BLOOD PRESSURE, HEMATOCRIT AND PLASMA PROTEIN LEVELS BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF 4 TIMES CONCENTRATED PLASMA OF BOTH DONOR AND CADAVER ORIGIN TO A PATIENT OVER A 25 DAY PERIOD

Date	Type of plasma or serum	Amount injected (gms. plasma in c.c. aq. dist.)	Blood pressure	Hematocrit	Plasma protein (falling drop method)
9-17-43	Donor plasma	8 gm. in 40 cc.	124/68	33	6.53
	15 minutes after administration		132/80	31	6.19
9-18-43	Cadaver plasma	8 gm. in 40 cc.	122/82	32	6.6
	15 minutes after administration		132/85	31	6.9?
9-20-43	Cadaver serum	8 gm. in 40 cc.	120/68	34	6.6
	15 minutes after administration		110/75	30	6.2
9-29-43	Cadaver plasma	8 gm. in 40 cc.	102/64	32	7.2
	15 minutes after administration		115/70	32	7.0
10- 6-43	Cadaver serum	1 gm. in 4 cc.	..	..	..
10-12-43	Cadaver plasma	8 gm. in 40 cc.	120/65	31	7.1
	15 minutes after administration		124/70	29	6.7

**Conclusions.** The collection, preparation and administration of dried plasma from cadaver blood is a practical procedure and is recommended during our present wartime emergency. After the war, the commercial houses could probably supply the nation's albumin or plasma needs from cadaver blood. Recommendations could easily be made for the selection of cadavers (cause of death, time of death, etc.) and for techniques of collecting and appropriately processing (into dried plasma or probably preferably, dried albumin) cadaver blood obtained at mortuaries or hospitals. The use of products of cadaver blood should be reconsidered by the medical profession of the United States and Great Britain.

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## ELECTROCARDIOGRAPHIC PATTERNS IN CARDIOVASCULAR SYPHILIS\*

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THE pathology and pathogenesis of cardiovascular syphilis are more completely understood today than when Lancisii<sup>26</sup> first showed that the aneurysms observed by Paré<sup>31</sup> were the result of the disease.<sup>5,10,22</sup> Apparently the spirochetes establish a bridgehead in the wall of the aorta, invade the aortic wall, and weaken it until dilatation or aneurysm results. There also may be a downward infiltration involving the aortic valve, causing ring dilatation and valvular deformity leading to aortic regurgitation. Because the sinuses of Valsalva are favorite locations for the syphilitic involvement, the mouths of the coronary arteries are often encroached upon and occasionally are completely obliterated by the syphilitic process. Secondary to the coronary ostial involvement, there is often interference with the blood supply of the heart which leads to damage to the myocardium ranging from mild fibrosis to infarction. This, and more particularly the aortic regurgitation, often lead to cardiac hypertrophy.

Previous authors are in accord that the electrocardiogram is unchanged in early cardiovascular syphilis,<sup>1,14,19,38,39</sup> but in the later stages when the coronary ostia and the aortic valves are involved, electrocardiographic abnormalities are encountered. Most of the reports<sup>1,6,12,14,17,19,20,37,39</sup> have consisted of a description of the deviations of the various individual components, especially QRS and T, although instances of A-V<sup>1,12,17,20,37</sup> and intraventricular block<sup>1,6,12,14,20,37</sup> have been noted. When left ventricular preponderance was diagnosed it was commonly done so on the basis of left axis deviation alone,<sup>14,17,27,37</sup> abnormalities of the S-T-T component being attributed to coronary insufficiency.<sup>20</sup> Stressing the significance of S-T-T changes in left heart strain, Barnes and Whitten<sup>4</sup> included 8 examples of left ventricular preponderance due to cardiovascular syphilis in their illustrative cases. Ashman and Hull<sup>2</sup> also noted the presence of S-T-T- changes in left ventricular hypertrophy due to syphilitic aortic regurgitation.

Only a few correlations of electrocardiographic changes with post-mortem findings have been reported.<sup>6,12,20,37</sup> It is the purpose of this paper to analyze the electrocardiograms in autopsied cases of cardiovascular syphilis in terms of special patterns which have recently been observed to occur under specific circumstances.<sup>3,23,24</sup> The analysis is based on a series of 30 consecutive necropsied cases. Such a

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TABLE 1.—CASES IN WHICH SYPHILIS WAS THE SOLE FACTOR LEADING TO CARDIAC ABNORMALITIES RESPONSIBLE FOR ELECTROCARDIOGRAPHIC CHANGES

Case	Anatomic changes		Heart weight (gm.)	Ventricular thickness (mm.)		Electrocardiographic patterns	Age (yrs.)	Blood pressure (mm. Hg)	Illustrated in Figures—
	Syphilitic	Associated		Left	Right				
1	1. Multiple aneurysms of arch of aorta 2. Aortitis 3. Aortic regurgitation	.....	350	19	8	Left ventricular preponderance, 2nd type	68	135/85	1 A
2	1. Aortitis 2. Aortic regurgitation 3. Both coronary ostia markedly stenosed	1. Healed mitral and aortic endocarditis	450	20	6	Left ventricular preponderance, mixed type	55	150/100	1 B
3	1. Aortitis 2. Aortic regurgitation 3. Both coronary ostia encroached upon	1. Myocardial fibrosis	435	25	6	Left ventricular preponderance, mixed type	53	150/40	1 C
4	1. Aortitis 2. Aneurysm of innominate artery 3. Aortic regurgitation 4. Right coronary ostium encroached upon	1. Mild coronary sclerosis; all lumens patent	730	18	5	Left ventricular preponderance, mixed type	64	170/40	1 D
5	1. Aortitis 2. Aortic regurgitation 3. Right coronary ostium completely obliterated; left narrowed	1. Myocardial fibrosis	550	14	3	Left ventricular preponderance, 2nd type; progressing to intraventricular block of 1st indeterminate type	47	180/50	1 H
6	1. Aortitis 2. Aortic regurgitation 3. Both coronary ostia markedly narrowed	1. Slight coronary sclerosis; all lumens patent 2. Myocardial fibrosis 3. Infarction of anterior wall of left ventricle	400	24	3	Pattern associated with infarction of anterior wall of left ventricle; Lead 1, atypical	63	110/50	1 E
7	1. Aortitis 2. Aneurysm of ascending aorta 3. Marked narrowing of left coronary ostium	1. Slight coronary sclerosis; all lumens patent 2. Infarction of apex of left ventricle	575	17	2	Pattern associated with infarction of anterior wall of left ventricle (QRS 2 and 3 down and larger than upright QRS I)	50	146/42	1 F
8	1. Aortic regurgitation 2. Aneurysm of sinus of Valsalva and arch of aorta 3. Aortic regurgitation 4. Right coronary ostium completely obliterated; left narrowed	1. Slight coronary sclerosis; all lumens patent 2. Early infarction of septum	580	17	2	Intraventricular block of indeterminate S type; A-V block	44	100/50	1 G

TABLE 2.—CASES IN WHICH THE SYPHILITIC ANATOMIC ABNORMALITIES WERE COMBINED WITH THOSE OF OTHER ETIOLOGIC FACTORS TO PRODUCE THE ELECTROCARDIOGRAPHIC PICTURE

Case	Syphilitic	Anatomic changes	Associated	Heart weight (gm.)	Ventricular thickness (mm.)		Electrocardiographic patterns	Age (yrs.)	Blood pressure (mm. Hg)	Illustrated in Figures—
					Left	Right				
9	1. Aortitis 2. Aneurysm of ascending aorta 3. Aortic regurgitation 4. Both coronary ostia markedly encroached upon	1. Myocardial fibrosis 2. Old pyclophritis and arteriosclerotic changes of kidneys		700	20	8	Left ventricular preponderance, concordant type	65	210/48	2 A
10	1. Aortitis 2. Aortic regurgitation 3. Moderate stenosis of both coronary ostia	1. Coronary sclerosis with narrowing of lumen 2. Old healed posterior wall infarction 3. Myocardial fibrosis 4. Acute verrucous superimposed on an old fibroplastic mitral and aortic endocarditis		650	15	4	Left ventricular preponderance, 2nd type	47	176/66	2 B
11	1. Aortitis 2. Aneurysm of ascending aorta 3. Aortic regurgitation 4. Narrowed rt. coronary ostium	1. Slight coronary sclerosis; all lumens patent 2. Myocardial fibrosis 3. Nephrosclerosis of arteriolar variety		710	21	4	Left ventricular preponderance, 2nd type; auricular fibrillation	66	250/110	2 C
12	1. Aortitis 2. Aortic regurgitation	1. Slight coronary sclerosis; all lumens patent 2. Arteriosclerotic changes of aortic valve 3. Myocardial fibrosis 4. Acute interstitial nephritis and early nephrosclerosis of arteriolar variety 5. Acute vegetative endocarditis of mitral and tricuspid valves		675	16	2	Left ventricular preponderance, 2nd type; frequent auricular premature contractions	55	184/74	2 D
13	1. Aortitis 2. Aneurysm of ascending aorta 3. Aortic regurgitation	1. Myocardial fibrosis 2. Subacute glomerulonephritis		775	18	5	Left ventricular preponderance, mixed type	34	224/118	2 E
14	1. Aortitis 2. Aortic regurgitation	1. Marked coronary sclerosis with narrowing of the lumens, the right especially 2. Arteriosclerosis of aortic valve 3. Nephrosclerosis of arteriolar type 4. Old occlusion of left coronary artery 5. Old apical and septal infarction		550	10	4	Left ventricular preponderance, 2nd type	62	226/122	2 F
15	1. Aortitis 2. Marked narrowing of right coronary ostium	1. Myocardial fibrosis 2. Nephrosclerosis of arteriolar type		425	10	..	Intraventricular block of indeterminate S type; deep Q in CF <sub>2</sub> is suggestive of old anterior wall infarction	60	170/110	2 G

TABLE 2.—(Continued)

Case	Anatomic changes		Heart weight (gm.)	Ventricular thickness (mm.)		Electrocardiographic patterns	Age (yrs.)	Blood pressure (mm. Hg)	Illustrated in Figures— 2 H
	Syphilitic	Associated		Left	Right				
16	1. Aortitis 2. Aneurysm of ascending aorta 3. Aortic regurgitation	1. Moderate coronary sclerosis with narrowing of lumen 2. Myocardial fibrosis 3. Nephrosclerosis of arteriolar type	475	14	5	Intraventricular block of common type	50	154/80	
17	1. Aortitis 2. Aneurysm of sinus of Valsalva 3. Narrowing of right coronary ostium 4. Aortic regurgitation	1. Marked coronary sclerosis with narrowing of lumen, left especially 2. Old healed lateral wall infarction 3. Septal scar	600	15	6	Anterior wall pattern of myocardial infarction with septal involvement suggested by partial A-V block	57	144/44	2 I
18	1. Aortitis 2. Aortic regurgitation 3. Marked narrowing of both coronary orifices	1. Mild coronary sclerosis; all lumens patent 2. Myocardial fibrosis 3. Mitral endocarditis and stenosis 4. Congenital bicuspid aortic valve 5. Nephrosclerosis of arteriolar type	550	21	5	Non-specific abnormalities including T-wave changes in chest lead during an attack of pain	60	140/60	2 K
19	1. Aortitis 2. Aneurysm of ascending and descending aorta 3. Marked narrowing of right coronary ostium	1. Moderate sclerosis of both coronary arteries with narrowing of lumens 2. Myocardial fibrosis 3. Embrysepsia 4. Mildly tuberculous including myocardium	350	12	1	Non-specific abnormalities including low voltage	59	128/80	2 J

TABLE 3.—CASES IN WHICH SYPHILIS WAS MERELY COINCIDENTAL AND THE ELECTROCARDIOGRAPHIC FINDINGS WERE ASSUMED TO OTHER NON-SYPHILITIC ETIOLOGIC FACTORS

20	1. Aortitis	1. Moderate coronary sclerosis, lumens narrowed 2. Nephrosclerosis of arteriolar type	575	13	7	Left ventricular preponderance, concordant type	59	174/120	3 L
21	1. Aortitis 2. Multiple aneurysms of aorta	1. Marked sclerosis of both coronary arteries with narrowing of lumens 2. Nephrosclerosis of arteriolar type 3. Myocardial fibrosis	425	13	3	Left ventricular preponderance, 2nd type	61	170/90	3 J
22	1. Aortitis	1. Mild coronary sclerosis, lumens patent 2. Nephrosclerosis of arteriolar type	675	20	6	Left ventricular preponderance, concordant type	69	190/100	3 C

1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aneurysms of ascending aorta	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. Aortitis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
2. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
3. Aortic regurgitation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
4. Narrowing of both coronary ostia</																														

### Vitamin B deficiency.



study might establish any electrocardiographic patterns or changes peculiar to syphilis and would delineate the respective rôle played by the individual syphilitic lesion as well as that of any other associated etiologic factor present. For this purpose, the cases were subdivided into 3 categories: (1) Cases in which syphilis was the sole factor leading to cardiac abnormalities responsible for the electrocardiographic changes (Table 1). (2) Cases in which the syphilitic anatomic abnormalities were combined with those of other etiologic factors to produce electrocardiographic changes (Table 2). (3) Cases in which syphilis was merely coincidental and the electrocardiographic changes were primarily ascribed to other non-syphilitic findings (Table 3).

TABLE 4.—SUMMARY OF ANATOMIC AND ELECTROCARDIOGRAPHIC FINDINGS

Case No.	Anatomic changes										Electrocardiographic patterns					
	Cardiac hypertrophy	Aortic regurgitation	Coronary aortic stenosis		Coronary sclerosis	Myocardial infarction	Myocardial fibrosis	Hypertension*	Misc. concomitant factors	Left ventricular preponderance	Right ventricular preponderance	Infarction patterns	Intraventricular block	A-V block	Other non-specific abnormalities	
			R	L												R
1	L/r	x	..	..	..	..	..	..	..	x	..	..	..	..	..	
2	L/r	x	3+	3+	..	..	..	..	..	x	..	..	..	..	..	
3	L/r	x	1+	1+	..	..	..	x	..	x	..	..	..	..	..	
4	L/r	x	2+	..	..	..	..	..	..	x	..	..	..	..	..	
5	L	x	4+	3+	..	..	..	x	..	x	..	..	x	..	..	
6	L	x	3+	3+	..	..	x	x	..	..	..	A	..	..	..	
7	L	x	..	3+	..	..	x	..	..	..	..	A	..	..	..	
8	L	x	4+	3+	..	..	x	..	..	..	..	..	x	x	..	
9	L/r	x	3+	3+	..	..	..	x	x	..	x	..	..	..	..	
10	L/r	x	2+	2+	1+	1+	x	x	..	x	..	..	..	..	..	
11	L/r	x	1+	..	..	..	..	x	x	..	x	..	..	..	..	
12	L	x	..	..	..	..	..	x	x	..	x	..	..	..	x	
13	L/r	x	..	..	..	..	..	x	x	..	x	..	..	..	x	
14	L/r	x	..	..	2+	1+	..	..	x	..	x	..	..	..	..	
15	L	..	3+	..	..	3+	x	x	x	..	..	..	x	..	..	
16	L/r	x	..	..	1+	1+	..	x	..	..	..	..	x	..	..	
17	L/r	x	1+	..	1+	2+	x	..	..	..	..	A	..	x	..	
18	L/r	x	3+	3+	..	..	..	x	..	x	..	..	..	..	x	
19	L/r	..	3+	..	1+	1+	..	x	..	x	..	..	..	..	x	
20	L.R.	..	..	..	1+	1+	..	..	x	..	x	..	..	..	..	
21	L	..	..	..	2+	2+	..	x	x	..	x	..	..	..	..	
22	L/r	..	..	..	..	..	..	..	x	..	x	..	..	..	..	
23	L.R.	..	..	..	3+	2+	x	..	x	x	..	x	..	..	..	
24	L	..	..	..	2+	2+	..	..	..	x	..	..	..	..	x	
25	R	..	..	..	..	..	..	x	..	x	..	x	..	..	..	
26	L	x	..	..	3+	3+	x	..	..	x	..	P	x	..	..	
27	L	..	..	..	2+	2+	x	..	..	..	..	..	..	..	x	
28	..	..	..	..	..	..	..	..	x	..	..	†	..	..	..	
29	L/r	x	2+	2+	..	..	..	..	x	..	..	..	..	..	..	
33	..	x	1+	1+	..	..	..	..	x	..	..	..	..	..	x	

L = Left ventricular hypertrophy. R = Right ventricular hypertrophy. L/r = Predominantly left ventricular hypertrophy. L.R. = Both ventricles hypertrophied, neither predominantly. A = Anterior wall. P = posterior wall.

\* Based on clinical evidence.

† Acute diffuse pericarditis.

The electrocardiograms of these cases are illustrated in Figures 1 to 3.

The patterns upon which this study was based are: (a) a normal record and the normal variants, left axis shift and right axis shift; (b) non-specific abnormalities including intraventricular block of the

various types, A-V block, auricular fibrillation and other arrhythmias; and (c) the specific abnormal patterns of preponderance and infarction. The criteria for these patterns have been described in detail elsewhere.<sup>3,7,8,21,23,24</sup>

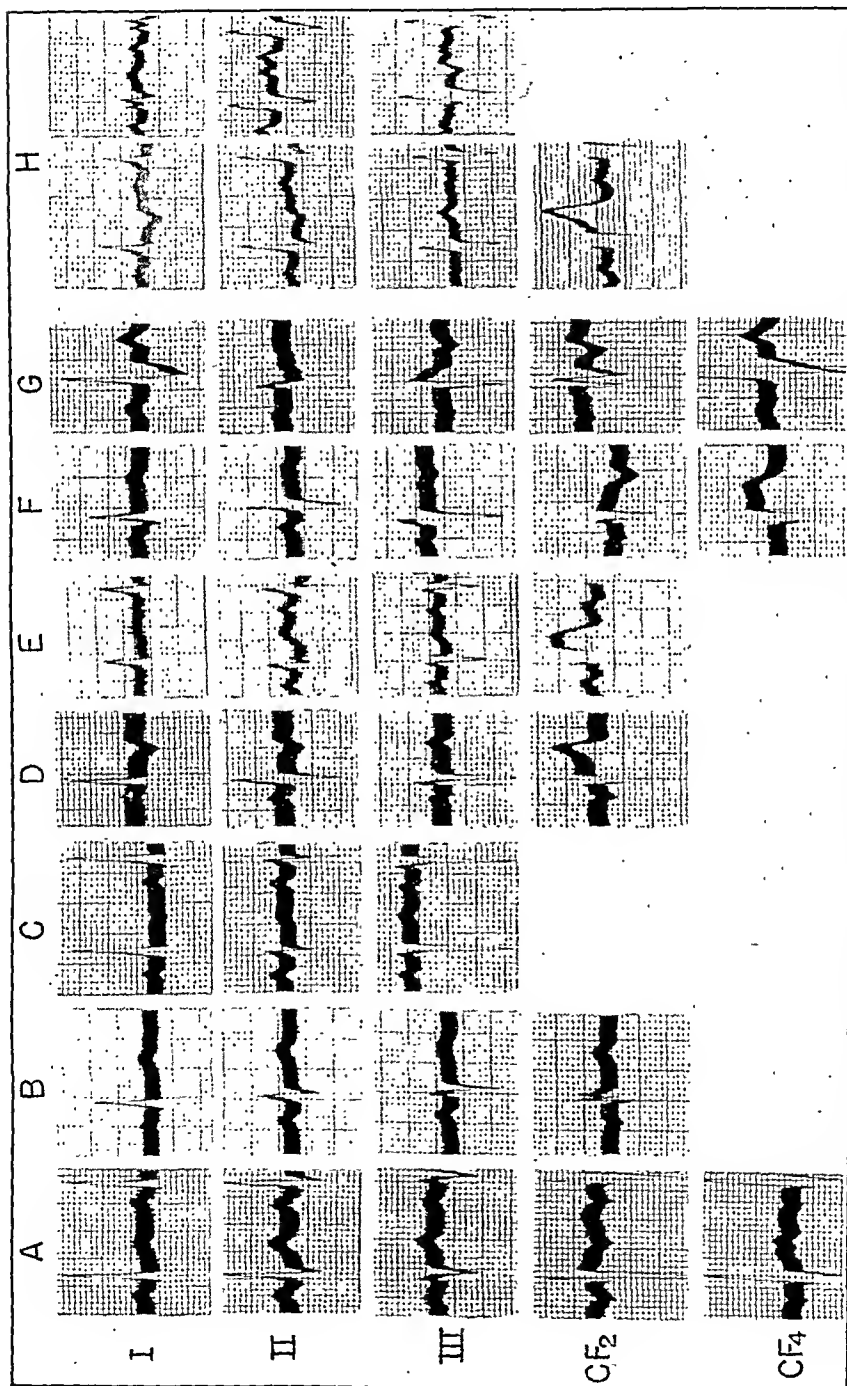


Fig. 1.—Segments of the electrocardiograms of the 8 cases in which syphilis was found at necropsy to be the sole factor leading to cardiac abnormalities responsible for electrocardiographic changes. The essential findings of these cases are summarized in Table 1. Segment A is Case 1; B, Case 2; C, Case 3; D, Case 4; E, Case 6; F, Case 7; G, Case 8; H, Case 5 (the 2 records were taken 1 month apart).

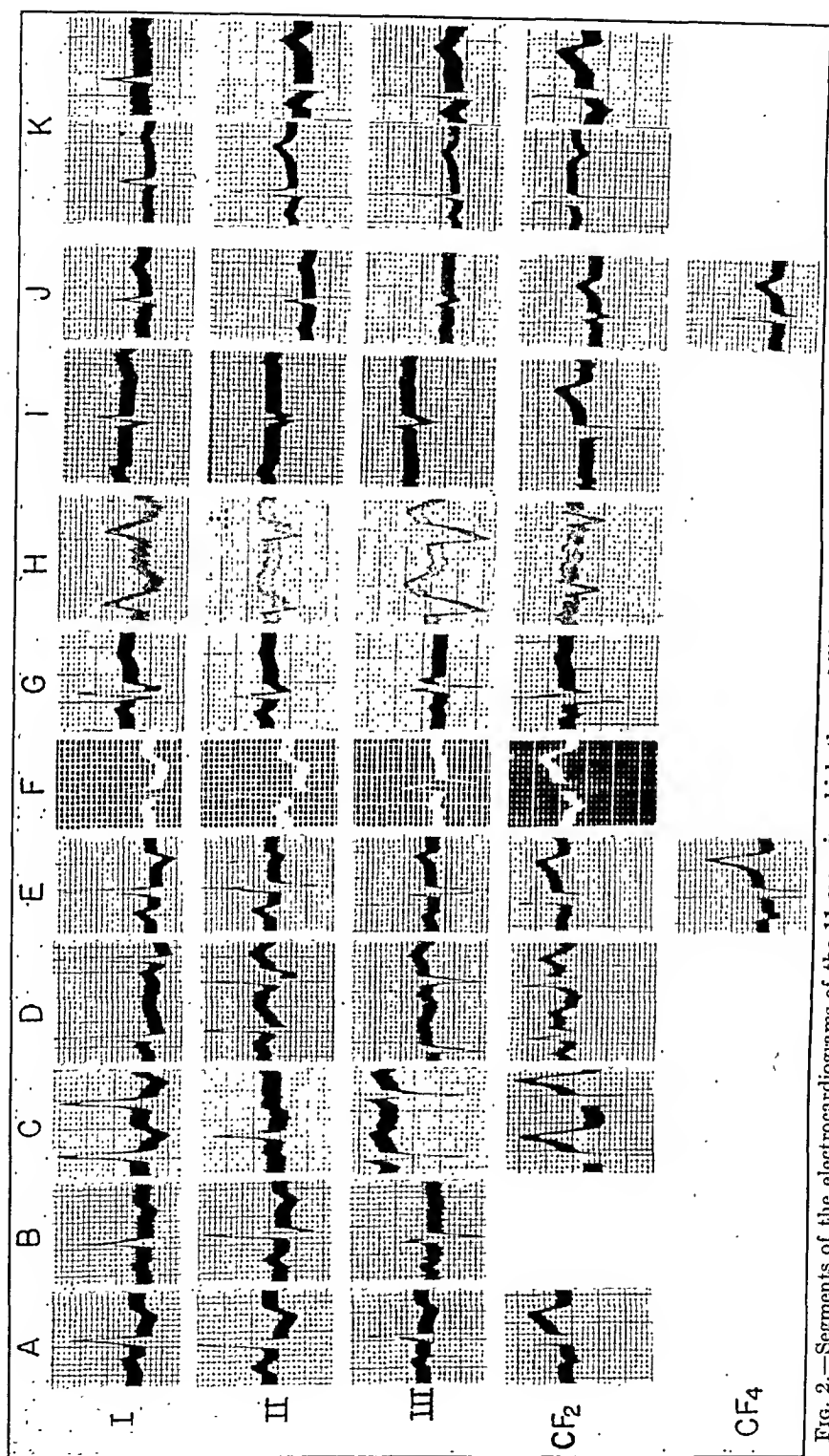


Fig. 2.—Segments of the electrocardiograms of the 11 cases in which the syphilitic anatomic abnormalities were combined with those of other etiologic factors to produce the electrocardiographic picture. The essential findings of these cases are summarized in Table 2. Segment A is Case 9; B, Case 10; C, Case 11; D, Case 12; E, Case 13; F, Case 14; G, Case 15; H, Case 16; I, Case 17; J, Case 19; K, Case 18 (the 2 records were taken 1 week apart).

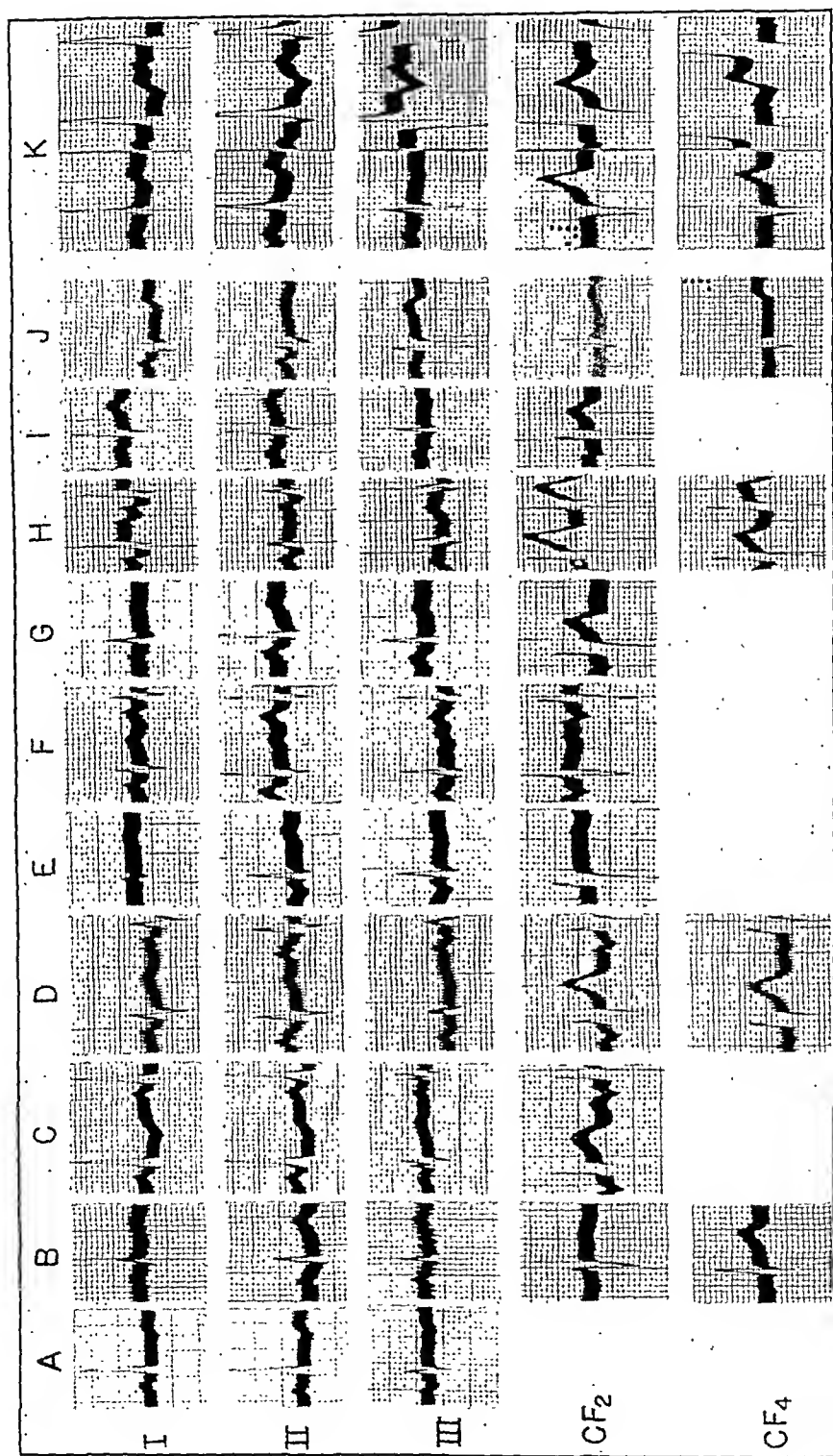


FIG. 3.—Segments of the electrocardiograms of the 11 cases in which syphilis was merely coincidental and the electrocardiographic changes were ascribed to other non-syphilitic etiologic factors. The essential findings of these cases are summarized in Table 3. Segment A is Case 20; B, Case 30; C, Case 22; D, Case 23; E, Case 24; F, Case 25; G, Case 27; H, Case 28; I, Case 29; J, Case 21; K, Case 26 (the 2 records were taken 11 months apart).

Left ventricular preponderance pattern (first, second, mixed and concordant types) indicates strain on the left heart and is found in conditions causing hypertrophy of the left ventricle. The pattern of right ventricular preponderance indicates strain on the right heart and is found in conditions resulting in hypertrophy of the right ventricle.

Anterior and posterior wall infarction patterns are associated with recent infarcts of the anterior-apical and posterior-basal regions of the left ventricle, respectively.

The occurrence of the various patterns with a survey of the various etiologic factors in the individual cases is summarized in Table 4. Therein, syphilitic coronary ostial stenosis was graded 1+ when slight, 2+ when moderate, 3+ when marked and 4+ when complete. Hypertension was considered to be a contributory factor when the systolic pressure was over 150 mm. Hg, when the diastolic pressure was over 100, and when there were present anatomic renal changes in the form of nephrosclerosis of the arteriolar variety, glomerulonephritis or pyelonephritis. When the aortic regurgitation was marked, a somewhat lower level of the diastolic pressure was not considered to be evidence against hypertension, if anatomic renal changes were found of a degree sufficient to lead to hypertension. Coronary sclerosis was considered to be a factor when some evidence of narrowing of the coronary arterial lumens was present. It was graded 1+ when moderate sclerosis with some stenosis was present, 2+ when the stenosis was severe and 3+ when the occlusion was complete.

**Results.** All 30 of the hearts and aortas in this series were found to be abnormal at postmortem. Twenty-nine of them were represented by abnormal electrocardiograms, of which 21 had specific patterns and 8 showed non-specific abnormalities. One electrocardiogram was normal.

There were 9 instances of myocardial infarction. Three of them, located on the anterior or lateral walls of the left ventricle, were represented by anterior wall patterns and a fourth anterior wall infarct by intraventricular block of indeterminate type.<sup>9</sup> One posterior wall infarct was represented by a posterior wall pattern, and one old posterior wall infarct showed left ventricular preponderance in the electrocardiogram; no chest leads had been taken in this case. One recent infarct of the septum showed A-V and intraventricular block. Finally, 2 infarcts of the lateral wall occurred in which no electrocardiograms were taken after the episode.

Of the 16 cases with syphilitic coronary ostial involvement, 7 showed electrocardiographic patterns of left ventricular preponderance and autopsy evidence of hypertrophy of the left ventricle; 3 showed anterior wall infarction patterns and infarcts were present at autopsy. One showed intraventricular and A-V block associated with an early septal infarct, 1 showed intraventricular block of the indeterminate S type, 3 had other non-specific abnormalities, and 1 showed a normal curve.

Aortic regurgitation was present in 19 cases. The electrocardiogram in 11 of these showed left ventricular preponderance and necropsy revealed left ventricular hypertrophy; 3, with myocardial infarcts, showed infarction patterns; 4 had non-specific abnormalities; and 1 was normal.

There were 28 cases of cardiac hypertrophy. The criteria for hypertrophy were based on findings in excess of the values given by Saphir<sup>34</sup> for normal hearts. The standards for left ventricular hypertrophy were heart weights in excess of 300 gm. and 250 gm. in the male and female, respectively, associated with left ventricular thickness of over 10 mm. The standard for right ventricular hypertrophy was a right ventricular thickness of over 3 mm. with similar heart weights.

In 25 of the 28 cases of cardiac hypertrophy, the left ventricle was involved, solely or predominantly. Left ventricular preponderance was present in the electrocardiograms of 14 of these cases, infarction patterns associated with infarcts were present in 3. Intraventricular block was present in 3, other non-specific abnormalities were found in 4 and a normal electrocardiogram occurred in 1 of these. In 2 of the cases in which the left and right ventricles were both hypertrophied to an approximately equivalent extent, 1 was represented by a left and the other by a right ventricular preponderance. In 1 case of hypertrophy of only the right ventricle, the electrocardiogram revealed right ventricular preponderance.

The chief concomitant factors encountered were coronary sclerosis, which was of sufficient degree in 12 cases to affect the electrocardiogram, and hypertension, which was present in 10 of the cases. Mitral endocarditis (rheumatic), bronchiectasis, tuberculosis, emphysema, a stab wound of the heart, vitamin B deficiency were also encountered and found to influence the electrocardiogram.

**Discussion.** The specific electrocardiographic patterns occurring most frequently in this series were left ventricular preponderance in 15 cases, and anterior wall infarction in 3 cases. Two of the 3 anterior wall patterns were associated with recent infarction due to syphilitic coronary ostial stenosis alone (Cases 6 and 7). The 3rd (Case 17) was due to a combination of syphilitic and coronary sclerotic processes.

The incidence of 9 myocardial infarctions in this series is high. A survey of the literature of the past 10 years revealed reports of only 12 cases of anatomically demonstrated myocardial infarction due solely to syphilitic coronary ostial stenosis<sup>11,12,13,29,34</sup> and in none of them were electrocardiograms shown or described. In 3 of the cases in the present series (Cases 6, 7 and 8) the infarct was on a purely syphilitic basis; in 3 others (Cases 10, 15, 17) the infarct was due to syphilitic stenosis and coronary sclerosis combined; and in the remaining 3 (Cases 23, 26, 27) the infarct was due to coronary sclerosis alone.

The frequency of coronary sclerosis complicating coronary ostial stenosis is one reason infarction attributable to syphilitic ostial encroachment, alone, is relatively rare. This, however, is not the sole

explanation. Usually encroachment of the coronary orifices by syphilis is such a gradual and slowly progressive process that the collateral circulation is able to keep pace with the occlusive mechanism. It is only in those cases in which this fails that infarcts develop. Apparently this latter circumstance is the more unusual in syphilis. In 1 case a coronary embolus arising from the wall of a syphilitic aorta resulted in an anterior wall infarction pattern, the patient succumbing too soon, however, for the characteristic morphologic changes of infarction to appear at postmortem examination.<sup>32</sup>

The second of the specific patterns frequently found, left ventricular preponderance, was present in 15 cases. There were 8 examples of the second type, 4 of the mixed and 3 of the concordant types. There were no instances of left preponderance of the first type in which axis deviation occurred without S-T-T abnormalities. This and the absence of axis deviation in one-fifth of the cases emphasizes again the importance of the S-T-T changes in the recognition of the preponderance pattern. Syphilitic lesions, aortic regurgitation, and coronary ostial stenosis were responsible in whole or in part for the left ventricular strain in 11 of the cases.

Aortic regurgitation operates by increasing the work of the left ventricle. It causes dilatation and eventually hypertrophy of that chamber. Syphilitic coronary ostial stenosis<sup>12,34</sup> has also been found to cause moderate hypertrophy, especially of the left ventricle, although usually not to the extent found in regurgitation. The two lesions were found to occur together so frequently that the rôle of each was difficult to ascertain. In Case 1, however, the hypertrophy of the left ventricle and the left ventricular preponderance could be attributed to syphilitic aortic regurgitation, *per se*.

The frequency of the specific anterior wall infarction and left ventricular preponderance patterns is greatest among the cases in which syphilis was the sole etiologic factor (Table 4). In the cases in which syphilis and other factors were combined, more non-typical patterns appear. Non-specific abnormalities occur with greatest frequency in the group of cases in which syphilis was merely a coincidental finding as far as the electrocardiographic contour was concerned.

Before the electrocardiogram in syphilitic cardiovascular disease can be properly evaluated, the extent and effect of concomitant disease factors must be determined, since they may produce similar findings, and in some cases superimpose typical patterns of their own. Thus hypertension and arteriosclerosis<sup>15,18,25,30,36</sup> which have been found to produce cardiac hypertrophy, caused the left ventricular preponderance patterns in Cases 20, 21, 22 and combined with syphilitic aortic regurgitation to produce them in Cases 9 to 14. Coronary sclerosis leading to posterior wall infarction dominated the electrocardiogram in Case 26 which had formerly shown a left ventricular preponderance pattern attributable in part to syphilitic aortic regurgitation. Mitral stenosis might have counteracted the tendency of syphilitic aortic regurgitation and coronary ostial stenosis to produce left preponder-

ance pattern in Case 18, and resulted in a right ventricular preponderance pattern in Case 25.

Of the various individual syphilitic lesions, it is apparent that syphilitic aortitis in itself does not affect the electrocardiogram. None of the 13 instances of aneurysm of the aorta and innominate arteries could be demonstrated to affect the electrocardiogram either because of situation or complication. There have been a few instances of complications of aneurysms recorded, however, such as an increase in the size and the position of the aneurysm which caused a change in position of the heart with resultant axis deviation,<sup>16</sup> an instance of aortic aneurysm rupturing into the pulmonary artery,<sup>37</sup> or into the pericardial sac<sup>35</sup> giving rise to a hemopericardium and an electrocardiographic pattern of acute diffuse pericarditis.

The rôle of syphilitic aortic regurgitation in the production of left ventricular preponderance patterns has been discussed above.

Coronary ostial stenosis may produce the specific pattern of left ventricular preponderance by leading to left ventricular hypertrophy or it may cause myocardial fibrosis for which there is no specific pattern. As the specialized conducting tissues of the heart are deprived of their blood supply by this occlusive process, A-V and intraventricular block appear. Thus in Case 5, the left ventricular preponderance due to aortic regurgitation and coronary ostial stenosis was later replaced by intraventricular block when the blood supply to the bundle branch system was affected. Mahaim<sup>28</sup> recognized the relationship of syphilitic ostial occlusion to intraventricular block. It is possible, however, that massive increase in heart size with the resulting relative coronary insufficiency on occasion may lead in syphilis, as in other circumstances, to broadening of the QRS span.◊

When, in bilateral involvement of the coronary orifices, complete obliteration of the right coronary ostium was present, conduction defects appeared in Cases 5 and 8, while, when the left coronary ostium was markedly narrowed, infarction of the anterior wall type resulted (Cases 6 and 7).

Diffuse syphilitic myocarditis and gummatous lesions were not encountered in this series.

**Summary and Conclusions.** The electrocardiograms and postmortem findings of 30 cases of cardiovascular syphilis are presented and correlated in each case. For this purpose the electrocardiographic tracings were divided into several specific patterns, the criteria for which have been established.

All 30 of the hearts and aortas in this series were found to be abnormal at postmortem. In 8 the anatomic findings and resulting electrocardiographic tracings were entirely on a syphilitic basis. In 11 other cases, syphilitic anatomic abnormalities were combined with those of other etiologic factors to produce the electrocardiographic pattern. The remaining 11 cases manifested syphilitic lesions as coincidental findings; the electrocardiographic contours being ascribed to the non-syphilitic lesions.



Although no specific pattern was found to be pathognomonic of syphilitic cardiovascular disease, two specific patterns occurred frequently due in whole or in part to syphilis. There were 11 cases of left ventricular preponderance and 3 cases of anterior wall infarction pattern.

The frequency of left ventricular preponderance and anterior wall infarction patterns was greatest in the cases having syphilis only, whereas, non-specific electrocardiographic abnormalities appeared when concomitant disease elements were present and non-typical patterns predominated when the latter were dominant.

Uncomplicated syphilitic aortitis, *per se*, and uncomplicated aneurysms caused no electrocardiographic abnormalities.

Aortic regurgitation produced left ventricular hypertrophy, reflected by various types of left ventricular preponderance in the electrocardiogram.

Coronary ostial stenosis in some instances caused myocardial fibrosis which has no specific electrocardiographic pattern. Stenosis of the mouths of the coronary arteries was shown to contribute to the formation of cardiac hypertrophy, especially of the left ventricle, resulting in left ventricular preponderance patterns electrocardiographically.

When localized areas of the myocardium were deprived of their blood supply by coronary ostial encroachment, corresponding specific infarction patterns were present in the electrocardiogram. These were all of the anterior wall type in our series.

When the septum and conducting system were similarly deprived of their blood supply, the electrocardiogram showed A-V and intra-ventricular block.

While the absence of electrocardiographic change does not rule out cardiovascular syphilis, most cases of far-advanced syphilis have abnormal electrocardiograms.

The presence of the special patterns of left ventricular preponderance and anterior wall infarction in cases of cardiovascular syphilis uncomplicated by other diseases is a good index, respectively, of hypertrophy of the left ventricle and myocardial infarction of syphilitic origin.

We are grateful to Dr. O. Saphir for permission to use his postmortem findings in these cases as the basis of the study; to Dr. R. Langendorf for his suggestions and criticisms and to Dr. L. N. Katz, under whose guidance this work was done, for his advice in carrying out the study. Some preliminary data was gathered for this study by Dr. Harold Engle before he left for service with the Army Air Forces.

ADDENDUM:—In a report appearing after this communication was in press, Evan Jones and D. Evan Bedford (*Brit. Heart J.*, 5, 107, 1943) cited 12 necropsied cases. All of them had aortic regurgitation and 10 had coronary ostial stenosis resulting from syphilis. The electrocardiograms in 10 were abnormal. However, the electrocardiograms were not shown and the detailed findings in the individual cases were not given.

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## THE PATHOLOGIC CHANGES IN SULFADIAZINE INTOXICATION\*

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NUMEROUS experimental and clinical reports indicated the great therapeutic value of the new sulfonamide drug, sulfadiazine, in various bacterial infections. Furthermore, early clinical trial showed a lower

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degree of toxicity for this preparation, elaborated by Roblin and his co-workers, than with other sulfonamide compounds. The need for such a drug led to its widespread use in military and civilian practice. With this extensive application reports appeared in the literature of serious and sometimes fatal toxic effects. The majority of the clinical and pathologic studies stressed the urinary complications, particularly crystalluria and secondary mechanical renal trauma and blockage. Toxic damage to other organs or systems was recognized, also, but the incidence was much less and histopathologic examinations were infrequent because most patients recovered. The purpose of this report is to present the pathologic findings in 3 fatal cases, treated with sulfadiazine for streptococcic infections. In the first 2 patients severe organic damage was encountered; the 3rd case is presented as a control to illustrate the normal tissue reactions to sulfadiazine. Complete autopsies were performed.

**Case Studies.** CASE 1. V. K., white male, age 28; streptococcic nasopharyngitis, increasing toxemia, coma, cyanosis, fever up to 106.2° F., death on the 13th hospital day. This patient was admitted to hospital on Jan. 1, 1943. He complained of generalized aches and pains and sore throat of 1 weeks duration. Past personal history was negative, except for pneumonia in 1932 with uneventful recovery. Admission examination: temperature 102° F., pulse 104, respiration 22 per minute. The mucosa of the nasopharynx was hyperemic; the tonsils had been removed. Both anterior cervical lymph node chains were moderately enlarged. The chest examination revealed only a few coarse râles over the main bronchi.

A throat specimen was taken for smear and culture and routine upper respiratory treatment was instituted. Sulfadiazine was administered with an initial dose of 3 gm. followed by 1 gm. every 4 hours. Other treatment included bed rest, fluids, gargles and codeine cough syrup.

**Laboratory Reports.** *Throat cultures* positive for a non-hemolytic streptococcus on admission and on 2 subsequent examinations. *Urinalysis* negative on Jan 4 and 9, 1943. *Blood:* red cells 5,180,000, hemoglobin 16.7 gm., white cells 7800 (68% polymorphs, 30% lymphocytes, 2% eosinophils). *Chest Roentgen ray:* on Jan. 10, 1943: "No infiltration in either lung field. There is slight elevation of the right diaphragm."

**Clinical Course.** Within a few days after admission the patient's condition was much improved and an uneventful recovery was expected. However, on Jan. 9, 1943 a generalized purpuric skin eruption appeared. Sulfadiazine was stopped and fluids were forced. The 24 hour fluid intake on January 8 was 3100 cc., the urinary output 1050 cc. The temperature at this time was 102.6° F., pulse 102, respirations 23 per minute. A total of 45 gm. of sulfadiazine had been given up to the time of the eruption. During the next 36 hours the clinical course became stormy: the temperature rose to 106.2° F.; severe dyspnea, cyanosis, mental confusion and restlessness developed.

**Additional Laboratory Studies.** *Blood:* free sulfadiazine level 5.7 mg. per 100 cc.; white cell count 14,900 (93% polymorphs, 6% lymphocytes, 1% basophils); non-prot. N, urea N and sugar normal. *Icteric index* 11. *Urinalysis:* negative.

#### LEGENDS FOR FIGS. 1 AND 2.

FIG. 1.—Spleen. Two Malpighian corpuscles are seen. The lymphocytes around the central arteriole are almost completely absent. Cellular debris, nuclear fragments and infiltrating polymorphonuclear leukocytes are apparent in this region. (200 X.)

FIG. 2.—Liver. Marked fatty metamorphosis is observed and all parts of the lobule are affected. (60 X.)

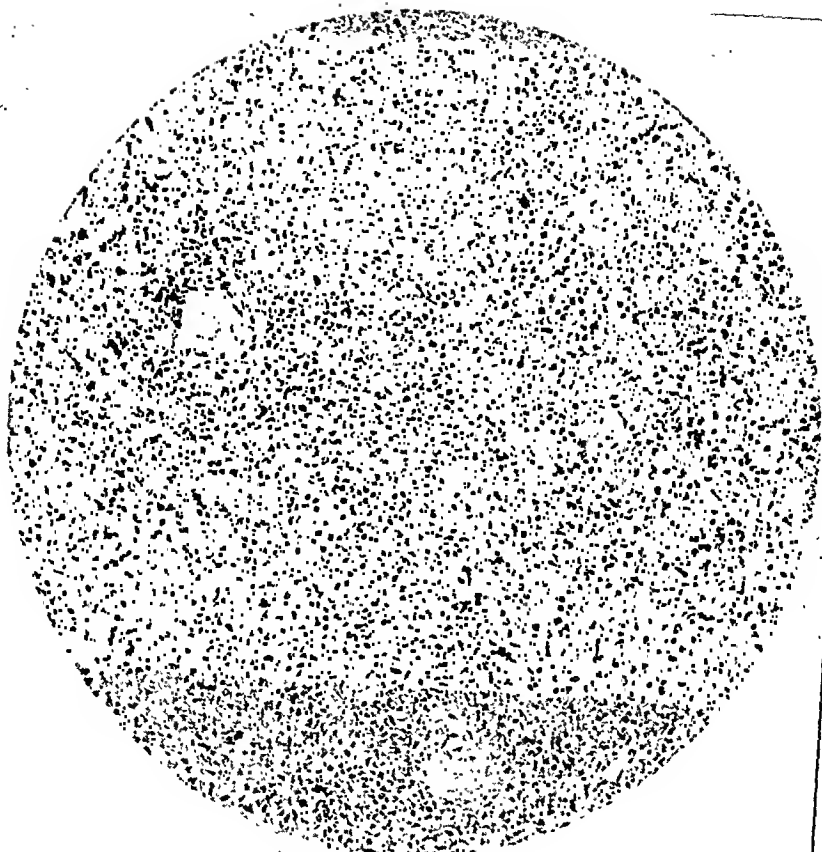


Fig.1

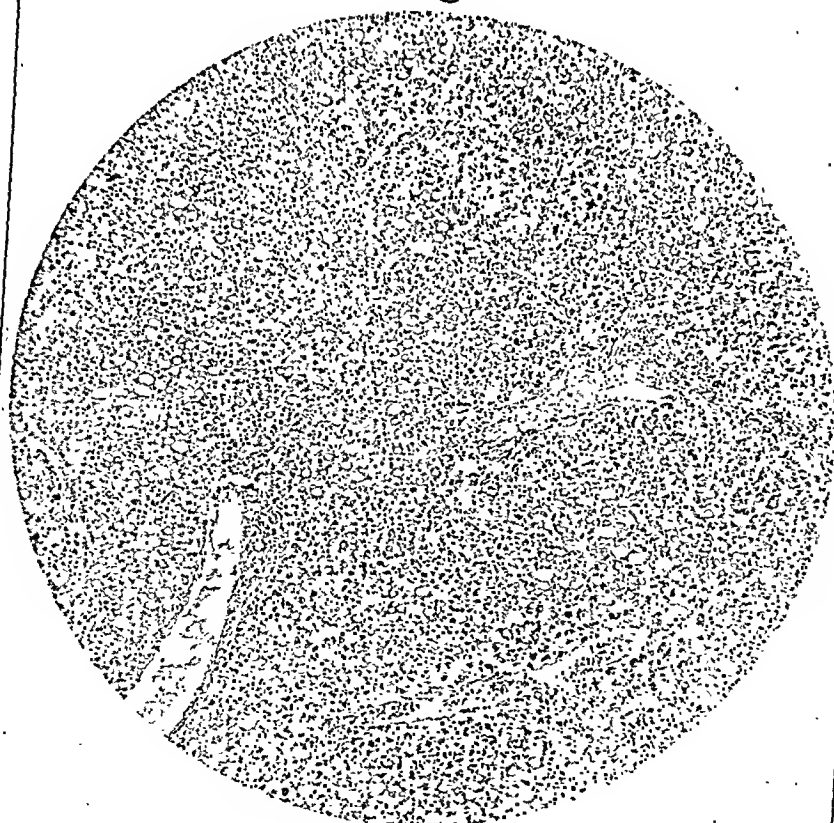


Fig.2

After consultation the sulfadiazine was resumed. It was believed that the mounting toxemia was secondary to the original upper respiratory infection rather than to sulfadiazine. The drug was readministered beginning on the afternoon of January 10 in 1 gm. doses every 4 hours; 14 gm. were taken. Five per cent glucose solution and blood plasma were given in 500 cc. doses on the same days. Oxygen was given by mask. The patient showed no response, lost consciousness and died on Jan. 13, 1943.

*Autopsy Abstract.* (2 hours after death):

**Gross.** The body was that of a well-developed, well-nourished young adult male, weighing 184 pounds and measuring 67 inches. The mucous membranes of the mouth were dry, crusted and extremely cyanotic. Numerous petechial and purpuric hemorrhages were present in the skin over the abdomen and lower extremities.

**Lungs:** The right weighed 435 gm., the left 480 gm. Dark purple congestion and slightly decreased crepitation were found in the dependent parts of both organs. The cut surface of each lung oozed frothy, bloody fluid; there was no consolidation. The bronchi contained a moderate amount of thick mucoid sputum. The blood-vessels were intact.

**Liver:** Weighed 2100 gm. The capsule was smooth, thin and transparent. The underlying parenchyma appeared yellow to orange with irregular patchy purple congestion. The cut surface was yellowish tan and contained faint purple patches. The vascular and biliary branches were normal.

**Spleen:** Weighed 315 gm., was moderately enlarged and of normal shape. The consistency was firm. The capsule was thin, purplish gray and wrinkled. The cut surface appeared reddish purple, scraped with difficulty; the follicles were prominent.

**Kidneys:** The right weighed 170 gm., the left 175 gm. The capsules were thin and stripped easily. The external surface was dark red. On section the cortex and pyramids appeared intact and reddish brown. No calculi were found in the pelvis or ureters.

**MICROSCOPIC.** (Formalin, paraffin, hematoxylin and eosin technique. Scharlach R stains were performed on frozen sections for fat.)

**Lungs:** The alveolar walls were markedly congested and edematous. Small focal areas of atelectasis with slight round cell and polymorph infiltration were apparent. There was no pneumonia. The vessels and bronchi were normal.

**Liver:** Marked fatty metamorphosis was present in the parenchymal cells. The fat was distributed in the form of coarse or fine droplets within the cell cytoplasm; in cells with coarse droplets the nuclei were pushed to the side and frequently appeared pale and vesicular. Some nuclei were enlarged, almost to giant proportions. The sinusoids were narrowed. Moderate hyperemia and polymorph infiltration were apparent in the periportal regions.

**Spleen:** Striking focal necrosis of the lymphoid cells was present in the Malpighian corpuscles (Fig. 1). This destruction of lymphoid collars varied from partial to complete, with abundant basophilic cellular debris, hemorrhages and occasional polymorphs in these areas.

**Lymph Nodes. Mesenteric:** The lymphoid follicles throughout the node were markedly necrotic; the changes were similar to the picture in the spleen. In these foci basophilic fibrinous debris, including pyknotic and fragmented nuclei, was abundant. The margins were hyperemic, infiltrated by polymorphs and contained occasional thrombosed vessels.

#### LEGENDS FOR FIGS. 3 AND 4.

FIG. 3.—Liver. Almost every liver cell in the field shows fatty metamorphosis. The cytoplasmic droplets vary in size, some coarse, many fine. (200X.)

FIG. 4.—Mesenteric lymph node. Marked necrosis with partial disappearance of the normal lymphocytes is noted. Nuclear pyknosis and fragmentation are severe. (200X.)

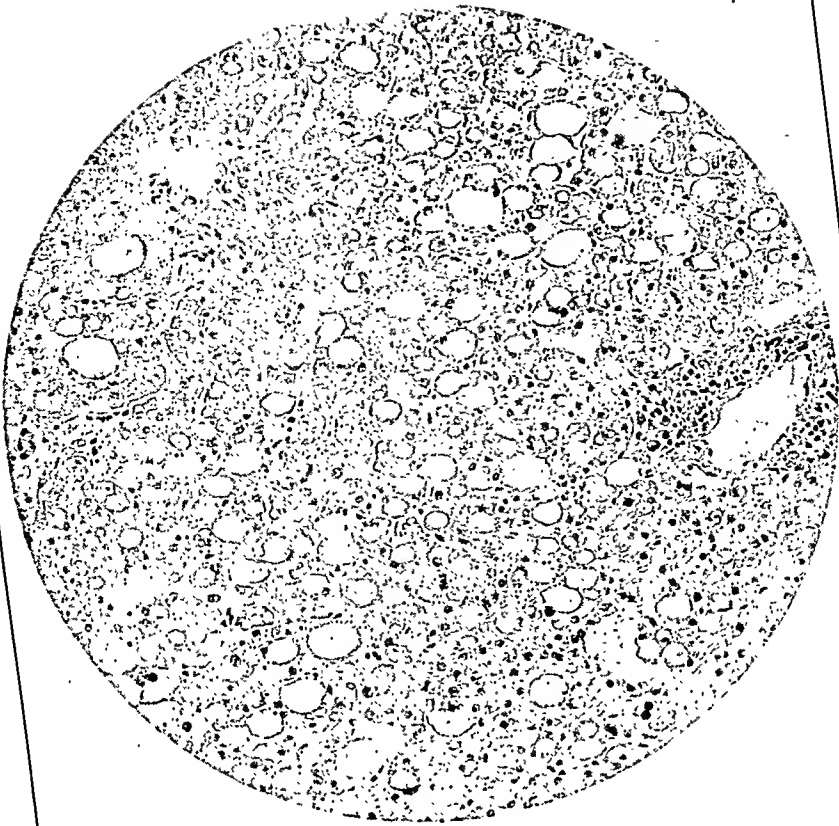


Fig. 3

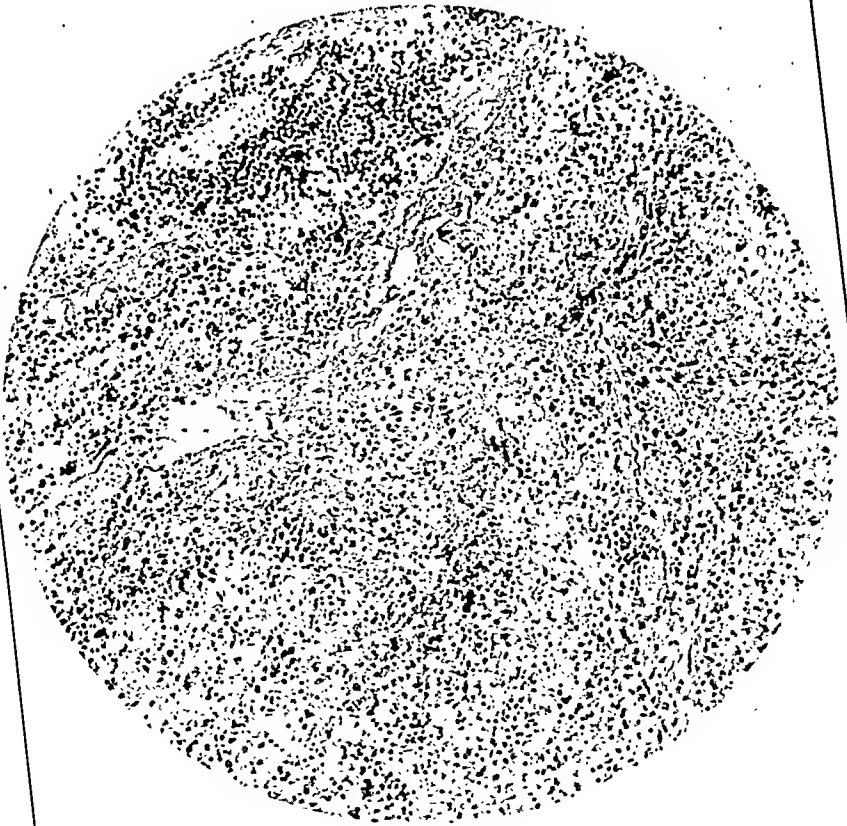


Fig. 4

*Bone Marrow. Ribs:* Hematopoiesis was very active. The megakaryocytes were numerous: as many as 12 were apparent in some high-power fields. No areas of necrosis were seen.

**ANATOMIC DIAGNOSES.** (1) Fatty metamorphosis of liver, severe, acute, toxic; (2) focal necrosis of spleen and mesenteric lymph nodes, moderate, acute, toxic; (3) acute passive congestion of lungs and abdominal viscera, severe.

**CASE 2.** L. M., white male, age 34; streptococcic nasopharyngitis, mounting toxemia, dyspnea, cyanosis, temperature to 105.8° F., coma, death on 13th hospital day. This patient was admitted to hospital on Jan. 1, 1943; he was a companion of Case 1. The complaints were sore throat, chills, fever and a tight feeling in the center of the chest, all present for 4 days. He had measles and scarlet fever in childhood. Admission examination: temperature 102° F., pulse 80, respirations 20 per minute. The nasopharyngeal mucosa was hyperemic and was covered with abundant mucopurulent exudate in the posterior pharynx. Anterior and posterior cervical lymph node chains were palpable. Heart was negative. The lungs were normal except for a few coarse râles over the main bronchi.

A throat specimen was taken for smear and culture and routine upper respiratory treatment was started. This included sulfadiazine, 3.0 gm. immediately, followed by 1 gm. every 4 hours. Fluid intake was encouraged and codeine cough mixture was administered as required.

*Laboratory Reports. Throat Culture:* Admission culture was positive for *S. viridans*; cultures on January 7 and 11 revealed non-hemolytic streptococcus. *Urinalysis* negative. *Blood:* January 2, red cells 5,720,000, hemoglobin 17.3 gm.; white blood cells 6600 with 52% polymorphs and 48% lymphocytes. *Chest Roentgen ray:* January 2, normal.

*Clinical Course.* Recovery appeared uneventful until Jan. 10, 1943, when dull pain developed in the right lumbar region and a skin eruption, identical with that in Case 1, was noted. Sulfadiazine was stopped and the fluid intake increased. A total of 33 gm. was ingested up to this time. The fluid intake on January 6 was 4525 cc.; urinary output 2925 cc.; on January 8 the intake was 4400 cc., output 1800 cc. During the next 72 hours the temperature rose steadily to 104° F. The patient became nauseated, vomited several times and subsequently developed cyanosis, dyspnea and restlessness.

*Laboratory Report at This Time. Blood:* Sulfadiazine level 4.3 mg. per 100 cc.; white cell count 11,900 (92% polymorphs, 8% lymphocytes); non-prot. N, urea N and sugar normal. Icteric index 11. *Urinalysis:* albumin 30 mg.; no casts or red blood cells; no crystals. *Chest Roentgen rays* repeated on January 10, 12 and 13, and showed no significant change from the early examination except for some elevation of the right leaf of the diaphragm.

Consultation was obtained. The unfavorable turn in the clinical course was interpreted as secondary to recrudescence of the original respiratory infection rather than from sulfadiazine toxemia. The sudden deterioration in the clinical condition of both patients seemed to coincide with the withdrawal of the drug. Therefore, sulfadiazine was resumed on January 10, with the dosage of 1 gm. every 4 hours, so that an additional amount of 16 gm. was taken. The treatment was supplemented with 5% glucose solution intravenously in 1000 cc. doses on January 10 and 11, 500 cc. of plasma intravenously on the same days and oxygen by mask. There was no response. The temperature continued to rise and on January 10 reached 105.4° F. The patient became comatose, and died on Jan. 13, 1943.

*Autopsy Abstract.* (12 hours after death):

**GROSS:** The body was that of a well-developed and well-nourished adult white male, weighing 156 pounds and measuring 65 inches. The nasopharyngeal mucous membranes were markedly cyanotic. Numerous fresh and fading petechial and purpuric hemorrhages, which varied in size from 1 to 3 mm. in diameter, were scattered in the skin and were most numerous over the anterior abdomen and lower extremities.

**Lungs:** The right weighed 610 gm., the left 325 gm. Small, scattered anthracotic deposits were present in the visceral pleura. The lung substance

was reddish gray anteriorly, with purplish mottling in the dependent portions. The latter were doughy and in some regions edematous. The cut surface oozed bloody fluid, was purplish red due to hypostatic congestion. The blood-vessels were normal. The bronchial mucosa appeared diffusely cyanotic.

*Liver.* Weighed 1760 gm., was slightly decreased in size and of normal shape. The capsule was thin and transparent. The parenchyma was yellowish brown with scattered purpuric hemorrhages. The consistency was soft and somewhat mushy. The parenchyma in the cut surface appeared yellowish to orange-brown and contained small purpuric hemorrhages. Mild passive congestion was distinguished in some parts. The vascular and biliary branches were normal.

*Spleen:* Weighed 425 gm., was enlarged in size and of normal shape. The capsule appeared thin, purplish gray and wrinkled. The cut surface was dark purple, firm, and scraped with some difficulty; the follicular markings were fairly distinct.

*Kidneys:* The right weighed 150 gm., the left 145 gm. The capsules were thin, stripped easily; the external kidney substance was reddish brown. The cut surface was moderately congested, the cortex reddish brown, the pyramids purplish brown. No concretions were found in the pelves or ureters. There were a few scattered petechiæ in the pelvic mucosæ.

*MICROSCOPIC. Lungs:* The alveolar walls were hyperemic, edematous and, in scattered fields, were mildly infiltrated by polymorphs and round cells. Some alveoli were collapsed; none of the alveolar lumens contained exudate. The arteries were normal. In several bronchi the lining epithelium was exfoliated and small foci of fibrinoid necrosis were visible in the submucosa. Round cells, polymorphs and nuclear débris were observed in the centers and margins of the necrotic foci.

*Liver:* All fields displayed disorganization of the normal structure and severe fatty metamorphosis. Almost all liver cells were affected. Some contained large fat droplets in the cytoplasm with eccentric displacement of the nucleus; in others the droplets were fine and distributed through the cytoplasm. A few areas were almost completely devoid of liver cells; here, only scattered nuclear shadows remained in an edematous background. The periportal regions were moderately infiltrated by round cells and polymorphs (Figs. 2 and 3).

*Spleen:* Widespread hyperemia and edema were present in the pulp. Focal fibrinoid necrosis was apparent in the lymphoid elements of the Malpighian bodies; in such necrotic zones disappearance or reduction in number of the lymphocytes was associated with much fragmented nuclear débris. Many lymphocytic nuclei were pyknotic; scattered polymorph infiltration was present along the margins of focal necrotic areas.

*Lymph Node—Mesenteric:* Focal and diffuse fibrinoid necrosis was observed. In such foci the cortical nodules were destroyed. Basophilic cellular débris and pyknotic and fragmented nuclei were prominent. The lymphocytes were decreased, absent in some fields, and the stroma hyperemic. Many small vessels were thrombosed. Polymorph infiltration was present throughout (Fig. 4).

*Kidneys.* The stroma was moderately hyperemic; the arteries appeared slightly thickened. The arterioles and glomeruli were intact. There was a small amount of pale staining, granular débris in the lumens of the proximal convoluted tubules; the tubular epithelium appeared cloudy and swollen.

*Bone Marrow.* The fatty stroma was moderately edematous and the cellular elements were composed predominantly of erythrocytes and granulocytes in all stages of development. The ratio appeared normal. Marked megakaryocyte activity could be distinguished.

**ANATOMIC DIAGNOSES.** (1) Fatty metamorphosis of liver, severe, acute, toxic. (2) Focal necrosis of spleen and mesenteric lymph nodes, moderate, acute, toxic. (3) Acute, passive congestion of lungs and abdominal viscera, severe. (4) Acute, focal, necrotizing bronchitis, mild.



CASE 3. H. R., white male, age 50; left peritonsillar abscess, cavernous sinus thrombosis, suppurative meningitis, brain abscesses, right hemiplegia, pneumonia, fever to 108° F., death on 13th hospital day. This case is reported as a control in the sense that it illustrates the relative harmlessness of the drug in a non-susceptible individual. The patient received intensive sulfadiazine treatment, much of it intravenously; the amount ingested and the free sulfadiazine blood level were much higher than in Cases 1 and 2.

The patient was admitted to hospital on Feb. 19, 1943, by transfer from a station hospital. The temperature was 103° F. and the patient was somewhat confused mentally. His complaints were chiefly difficulty in swallowing and pain in the right temple. There had been severe pain in the left side of the face on February 17, but this had subsided by the time of admission.

*Physical examination* revealed a left peritonsillar abscess, which was incised immediately and a large quantity of thick yellow pus was evacuated. On the following morning his condition seemed improved but on the next day inflammation and proptosis developed in the right eye and the temperature rose to 103° F. The diagnoses of cavernous sinus thrombosis and meningitis were made and oral and intravenous treatment with sulfadiazine was undertaken. The course was steadily retrogressive; hypostatic pneumonia developed on February 27, and right hemiplegia on March 2. Death occurred on March 3, 1943.

The sulfonamide treatment included: sulfathiazole 20 gm. in 5 days before entering Fitzsimons; sulfadiazine 62.6 gm. (17 gm. intravenously) in 11 days at Fitzsimons. The average blood level of free sulfadiazine was 15 mg. per 100 cc. (the lowest 4.4 mg.; the highest 20.4 mg.).

*Autopsy* (16 hours after death):

*Gross.* The body was that of a well-developed and fairly well-nourished middle-aged white male, weighing 135 pounds and 71½ inches in length. Generalized purple mottled discoloration was present in skin. The right orbital soft tissues were edematous, congested; there was a 0.7 cm. recent surgical incision at the upper internal angle. *Intracranial examination* revealed an acute generalized suppurative pachymeningitis and leptomeningitis; acute, suppurative thrombophlebitis in the left pterygoid plexus, in both cavernous sinuses, in the circular sinus, in the upper petrosals and in the right ophthalmic vein; multiple brain abscesses.

*Lungs:* The right weighed 860 gm., the left 815 gm. The visceral pleura was thin and glistening. Each lung appeared bright red anteriorly, purplish red in its dependent portion. The substance was edematous and airless posteriorly, doughy elsewhere. Emphysematous bullae were present along the fringes. The cut surface was congested, moist, and oozed bloody fluid on pressure. Dark purple areas were distinguished in the dependent regions. The bronchi contained a moderate amount of opaque white fluid, which appeared to be aspirated milk.

*Liver:* Weighed 2680 gm., was enlarged, soft and of normal shape. The capsule was thin and transparent. The parenchyma was mottled purplish red to purplish yellow. The cut surface was purplish red with irregular yellowish brown areas. Moderate passive congestion was seen.

*Spleen:* Weighed 235 gm., was moderately enlarged, soft and normal in configuration. The capsule was thin, wrinkled and purplish red. The cut surface was deep purple, scraped easily; the follicular markings were indistinct.

*Kidneys:* The right weighed 185 gm., the left 215 gm. The capsules were normal. The external surfaces were purplish red, contained scattered, small, depressed stellate scars and a few sharply demarcated, bright yellow adenomatous nodules, the largest 0.6 cm. in diameter. The cut surfaces of the kidneys were moderately hyperemic. No concretions were found.

*MICROSCOPIC. Brain and Meninges:* The leptomeninges were hyperemic and infiltrated by polymorphs and round cells. Exudate composed of fibrin, polymorphs and bacteria filled the subarachnoid space, particularly in the sulci and over the base. Many acute abscesses were scattered through the brain; their centers contained polymorphs, necrotic brain tissue, bacteria and cellular

débris; their margins were hyperemic with an occasional thrombosed vessel therein. Some vessels were ringed by polymorphs.

*Lungs.* Lobular inflammatory zones, some peribronchial, were present in the parenchyma. In these areas the alveolar capillaries were hyperemic and the bronchioles and alveolar lumens filled with fibrin, polymorphs and occasional red cells. The vessels elsewhere were not noteworthy.

*Liver:* Mild fatty changes were observed in the parenchyma; in some fields the liver cells adjoining the periportal region were involved; in others, those centrally located were affected. Moderate round cell infiltration was present in the interlobular trabeculae. Moderate passive congestion and cloudy swelling were observed.

*Spleen:* The Malpighian corpuscles appeared hyperplastic and the sinusoids were distended, congested and contained numerous polymorphs.

*Lymph Nodes—Peribronchial:* Acute hyperemia was noted; in some fields hemorrhage and polymorph response to the bronchiolar pneumonia could be distinguished. There was no necrosis of lymphoid tissue.

*Bone Marrow:* The stroma was hyperemic, edematous, in parts slightly hemorrhagic. Hematopoiesis was active; the erythromyeloid ratio appeared normal. The megakaryocytes were increased in number, with as many as 15 visible in some low-power fields.

*Kidneys:* The yellow nodules were cortical adenomata of adrenal type. The remaining parenchyma was hyperemic. Scattered small superficial scars were present in the surface zone of the cortex. The tubules contained small amounts of acid-staining granular débris.

**BACTERIOLOGIC EXAMINATION (POST MORTEM):** Cultures of exudate from the brain abscesses and meninges were positive for non-hemolytic streptococcus.

**ANATOMIC DIAGNOSES:** (1) Peritonsillar abscess, left, severe. (2) Acute, suppurative thrombophlebitis of left pterygoid plexus, of cavernous sinuses, of circular and upper petrosal sinuses, and of right ophthalmic vein. (3) Acute, suppurative leptomeningitis and pachymeningitis, severe. (4) Multiple brain abscesses, acute. (5) Mixed hypostatic and aspiration pneumonia, bilateral, severe. (6) Fatty metamorphosis of liver, mild, acute. (7) Multiple cortical adenomata of kidneys, bilateral adrenal type.

**Comment.** *Liver.* The accumulated evidence of toxic sulfonamide effects on the liver of susceptible individuals is so conclusive that no detailed reference to the literature is necessary. Sulfadiazine appeared to be less dangerous in this respect. Finland, Peterson and Goodwin<sup>5</sup> found no instance of hepatitis in 460 patients treated with it. In fact, 6 patients with severe liver damage in their series showed improved liver function during administration of sulfadiazine. On the other hand, Cantarow and Wirts<sup>2</sup> in a smaller group of 20 patients obtained results which pointed to hepato-cellular changes. They performed liver function tests during treatment with sulfadiazine and related drugs. No tissue studies were made.

The pathologic alterations described in Cases 1 and 2 consisted of marked diffuse fatty changes, which are designated usually as fatty metamorphosis. Such fatty alterations are observed in varying degrees in chronic alcoholism, benzene or chloroform poisoning and pulmonary tuberculosis. In the first 2 patients this was one of the important anatomic findings and its relation to the sulfadiazine treatment appears unquestionable. Neither patient received yellow fever vaccine previously. Undoubtedly the process caused impairment of liver function but never to the point of clinical jaundice.

In contrast to the liver damage in the first 2 patients, Case 3, H. R.,

showed only mild alteration which was of similar fatty character. Attention has been directed repeatedly by others to the lack of correlation between the dosage of sulfonamide drugs and the extent of toxic effects in susceptible persons. This is confirmed again in the cases here as shown by an analysis of the sulfadiazine dosage and blood levels. W. K. (Case 1) received 59 gm. of sulfadiazine over a period of 12 days; L. M. (Case 2) ingested 49 gm. in 9 days. The blood level was taken only once at the height of the treatment and was 5.7 mg. per 100 cc. in Case 1, 4.3 mg. in Case 2. H. R. (Case 3) was administered 20 gm. of sulfathiazole and 62.6 gm. of sulfadiazine (17 gm. sodium sulfadiazine intravenously) over 16 days and the blood level of free sulfadiazine ranged between 4.4 and 20.4 mg.; it averaged about 15 mg.

Case 3, H. R., received a greater amount of sulfonamide, most of it sulfadiazine, than either of the other 2 and his blood level was much higher. However, the liver changes were comparatively mild and probably reversible. The only significant difference in the clinical behavior of Case 3 was the absence of a skin eruption. No liver function tests were performed on any of the patients; the icteric index was slightly elevated in Cases 1 and 2.

*Spleen and Lymph Nodes.* Only a few studies are available concerning toxic changes in the spleen following the administration of sulfonamide drugs and the majority of these are experimental. The only report available to the author that concerns sulfadiazine is that of Maisel, McSwain and Glenn,<sup>8</sup> who studied tissue reactions in the dog. They found degenerative alterations in the "germinal centers" of the Malpighian bodies in nearly all of their animals. Davis, Harris and Schmeisser<sup>4</sup> observed focal necrosis, polymorph infiltration and nuclear fragmentation in the spleens of rats given sulfanilamide. Rake, Van Dyke and Corwin<sup>9</sup> described disorganization and disappearance of the Malpighian bodies in mice given 1% and 2% sulfathiazole diets. Lederer and Rosenblatt<sup>7</sup> saw similar changes in the spleen and lymph nodes of 4 human cases, who died after sulfathiazole treatment.

The changes described in Cases 1 and 2 were quite similar to those quoted above: the spleen displayed focal fibrinoid necrosis of the lymphoid elements in the Malpighian bodies. Nuclear fragmentation and cellular debris were marked and neutrophilic infiltration occurred throughout and in the margins of the necrotic foci.

The mesenteric lymph nodes showed similar focal, sometimes diffuse necrosis, which affected the cortical nodules particularly. The lymphocytes were decreased or absent in such areas and nuclear pyknosis and fragmentation were outstanding. This localization to mesenteric lymph nodes would seem, at first, to indicate an effect secondary to intestinal absorption of the sulfadiazine. However, the submucosal lymph follicles of the small intestine were unaffected.

Case 3 served as a control: there were no alterations in the lymph nodes except hyperemia and scattered hemorrhages but only the peribronchial nodes were available for examination. The spleen of this

patient presented changes characteristic of acute splenic tumor. This was very different from the picture in the other 2 cases.

**Bone Marrow.** Among the toxic effects of sulfadiazine upon the blood-forming organs, the depressing action on erythrocyte and granulocyte production assumes major importance. Such effects were described experimentally and clinically (Maisel, McSwain and Glenn,<sup>8</sup> Curry,<sup>3</sup> Satterthwaite<sup>10</sup> and Boyer.<sup>1</sup> Peripheral blood counts and bone marrow examinations revealed no comparable toxic action in the cases presented in this report.

Stimulation of megakaryocytes was apparent in the bone marrow of all 3 patients. No platelet counts were performed and the only significant clinical correlation was with the petechial and purpuric skin eruption in Cases 1 and 2. Whitehouse and Watkins,<sup>11</sup> and Gorham, Propp, Schwind and Climenko<sup>6</sup> described thrombocytopenic purpura following the use of sulfadiazine. Such reports indicate, therefore, that selective depressing effects may occur in susceptible individuals.

The possible relation of the splenic changes described above to the megakaryocyte activity is interesting, particularly since the spleen plays some rôle in thrombocytopenic purpura. No additional information is advanced in this report, however, and the findings in the spleen and bone marrow may be entirely independent.

**Summary.** 1. Three fatal cases of streptococcic infection are presented with complete autopsy findings. All of them received sulfadiazine treatment.

2. In 2 patients who developed a drug eruption, severe fatty metamorphosis of the liver and moderate focal fibrinoid necrosis of the spleen and mesenteric lymph nodes were observed. The 3rd patient showed no skin eruption, no necrosis of spleen or lymph nodes, and only mild fatty alterations were encountered in the liver.

3. A skin eruption during sulfadiazine treatment is of serious significance. *If a decision to continue with the drug is made following its appearance, then toxic effects should be expected and weighed against any possible beneficial influence on the original infection.*

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## CARCINOMA OF THE NASOPHARYNX

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PERIODICALLY there arises in the literature a review of this subject, but inquiry among recent medical graduates and others contacted in a large urban hospital reveals the general unawareness of carcinoma of the nasopharynx and its clinical syndrome. This lack of knowledge has undoubtedly been a factor in the poor control of this highly malignant disease. Every review in the literature presents an equally discouraging percentage of "cure." Many authors stress the element of late diagnosis as one of the reasons for this low percentage. Hence it seems desirable that various clinics should report their cases and the factors behind the therapeutic failures. We are reporting on 17 cases occurring at this hospital, 10 of which we have studied personally.

Martin and Blady<sup>5</sup> report that carcinoma of the nasopharynx comprises 2% of the malignant growths seen in the Head and Neck Clinic of the Memorial Hospital in New York. They also point out that this tumor occurs in a younger age group (mean age 45 years) than do tumors of the tongue, lip and mouth (56 to 58 years). It is interesting to note that several Chinese are included in many of the large series; Lenz<sup>4</sup> has 4 in a series of 63. Various authors report slightly different incidences of the following features:

1. *Cervical Adenopathy.* Kasaback<sup>3</sup> in 57 cases reports 77% showing cervical metastases on admission. Lenz<sup>4</sup> lists 36% in 61 cases. Furstenburg<sup>1</sup> states that 27.5% of his 41 cases showed cervical adenopathy on admission, but by the time the diagnosis was made 60% had palpable nodes in the neck.

2. *Nasorespiratory Symptoms.* Roughly 30% of cases in the literature give as a first symptom nasal obstruction, discharge or epistaxis.

3. *Neurologic Symptoms.* New<sup>7</sup> reports in 194 cases that 31% had paralysis of one or more cranial nerves. Other authors since have reported essentially similar figures. The most frequently involved cranial nerves are V, VI and IX, resulting in facial pain, diplopia and dysphagia respectively. These findings, plus headache, are explained by direct extension of the tumor into the middle fossa of the skull. Roentgenograms of the base of the skull show a typical melted-out appearance of the sphenoid and the petrous portion of the temporal bone. There are those<sup>5</sup> who claim that these findings are due to the erosive properties of the tumor once in the skull rather than by direct extension through the bone. They point out a possible pathway along the cartilaginous portion of the Eustachian tube up through the foramen lacerum accompanying the internal carotid artery (Fig. 9). Frequently it spreads a considerable way beneath the dura eroding the

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bone during its passage. Due to the involvement of the cervical sympathetic chain, a Horner syndrome may develop.

4. *Ear Symptoms.* As most of the tumors arise in or near the fossa of Rosenmüller, a number of auricular manifestations may be expected as early complaints. Martin and Blady<sup>5</sup> state that unilateral deafness was the first symptom in 10% of their cases. Tinnitus and a sensation of stuffiness are the other leading complaints. Later severe earache may be prominent.

5. *Duration of Symptoms.* Furstenburg<sup>1</sup> claims that the average duration of symptoms before diagnosis was 15 months. Precious time has been lost in the performance of many operations done on erroneous diagnoses. For example, tonsillectomies, mastoidectomies, and so forth were done in 32% of the cases of Hauser and Brownell.<sup>2</sup>

**Treatment.** Once the diagnosis is proven, irradiation to the limit of the skin and physical tolerance is the only accepted form of treatment.\* Martin and Blady<sup>5</sup> describe an ingenious radon applicator for topical application to the primary tumor in the nasopharynx. New<sup>7</sup> states that patients who were adequately treated with irradiation live 5 times as long as those who were not treated at all. One of the common faults in the handling of this condition has been to start treatment before the diagnosis has been established by biopsy. The tumor is so radiosensitive that any little irradiation may obliterate tissue favorable for biopsy, and the diagnosis is only secondarily established by removal of recurrent metastatic cervical nodes. The general opinion is that surgery is not indicated in the treatment of these tumors.

**Analysis of Material.** The accompanying chart of our 17 original cases shows numerous features confirming findings previously reported. Thus a younger mean age (38.5 years) is demonstrated than, for example, in malignancy of the mouth (56 to 58 years). Of our cases, 15 are males while only 2 are females. Once again the interesting incidence of Chinese in these cases becomes apparent, for 2 of our patients are of this race. Four negroes and 11 whites comprise the remainder. On admission every case had cervical lymph node metastases. In 11 of these patients the cervical lymph node enlargement was the original complaint. Three individuals complained primarily of epistaxis; 10 others developed it eventually. One referred to the ear in his original symptomatology, but 13 showed tinnitus or deafness in the course of their disease. In comparison with Needles<sup>6</sup> report of the occurrence of Horner's syndrome in 4 out of 35 cases, we had 1 in 17. Nine individuals showed signs of cranial nerve involvement. Roentgen rays of the base of the skull showed a melted-out defect of the middle fossa in 5 cases. In 2 cases there was clinical evidence of involvement of the cranial nerves without Roentgen ray evidence of damage to the base of the skull (Cases 9 and 17). The reverse was true in 1 case (Case 8). The average length of time from the onset of the first symptom to the time of diagnosis was 8.1 months. Of the

\* Irradiation should be given to each side of the face and neck with the portals extending about 1 inch above the zygomatic level and down to the base of the neck—approximate size of the field, 10 by 20 cm. Total fractional doses should average 3000 r, in air to each skin area. (Factors: 200 kv., 0.5 mm. Cu and 1 mm. Al filtration; 20 ma.; HVL 0.95 mm. Cu.) Occasionally additional treatment may be necessary.

17 cases, only 2 are alive (Cases 14 and 17). In the other cases the average length of life following the first symptom was 23.4 months. This figure represents the life span of a group of individuals admitted with cervical node metastases who received varying amounts of irradiation. This study, therefore, is a discussion of patients with late carcinoma of the nasopharynx. As in most other series, we are not in a position to offer comments on the early carcinoma of this region or any figures on the natural course of the disease unaffected by irradiation.

**Pathologic Anatomy.** Considerable confusion has centered about the term "lympho-epithelioma" coined by Schmincke<sup>8</sup> in 1921. The clinical syndrome produced by these tumors and their great radiosensitivity have led many to think of these as members of the malignant lymphomata or as representing an entirely new type of tumor. Schmincke pointed out that these tumors arose in a "lympho-epithelial" region, where normally considerable lymphoid tissue abounds, and that they were simply anaplastic carcinomas arising from the nasopharyngeal mucosa with many lymphocytes mixed in coincidentally. This is the commonly held view today. That these tumors are carcinomas may be readily seen from the accompanying photomicrographs. The same illustrations show that not all of them would fit the Schmincke description of "lympho-epithelioma." The tumors in our series tend to fall into 3 groups:

*Group I* (4 cases). This tumor consists of multiple, small, sharply demarcated but closely approximated islands of loosely packed cells. The individual cells contain rather small oval nuclei with one or two large nucleoli and a fairly coarse network making the nucleus appear dark. Abundant cytoplasm keeps the nuclei well separated. An occasional lymphocyte is seen between the cell groups. This microscopic picture is seen in Cases, 1, 6, 11 and 16 (Figs. 1 and 2).

*Group II* (2 cases). The second variety is made up of small single cords of tumor cells widely separated by an abundant stroma containing many lymphocytes and plasma cells. These cords, under low power, are strongly suggestive of anaplastic squamous carcinoma. The individual cells in the cords vary greatly. Some contain small dark staining oval nuclei while others contain large pale staining nuclei with large nucleoli. Cytoplasm in both types of cells is abundant. No prickly cells are seen. Such a microscopic picture is shown by Cases 4 and 14 (Figs. 3 and 4).

*Group III* (10 cases). This group of tumors embraces the largest number of our cases. It, in turn, is broken down into 3 subtypes. The basis of the subdivision is on the appearance of the general pattern, the cells being essentially similar.

*Subtype A* (5 cases). The tumor assumes the form of large invading solid blocks of tissue studded with cells containing uniformly huge pale-staining nuclei. These nuclei have exceedingly large nucleoli and a very delicate linen network. The cytoplasm of each cell appears to be merged with that of the next creating a pseudohomogeneous background for the nuclei. Only a few lymphocytes are seen nestled within this "block" of tumor tissue. Cases 2, 5, 8, 13 and 17 fall into this group (Figs. 5 and 6).

TABLE 1.—DATA ON 17 ORIGINAL CASES OF PRIMARY CARCINOMA OF THE NASOPHARYNX

Patient	Age	Sex	Color	First symptom or sign (date)	Months before diagnosis was made	Cervical nodes	Nasal and ear symptoms	Cranial nerve or intracranial symptoms	Skull involvement by Roentgen ray Note: metastases to lungs	Length of life
1. R. M.	26	M	W	Epistaxis (Nov. 1923)	12	Yes	Epistaxis	...	...	Died July 22, 1926 (32 mos.)
2. C. S. L.*	56	M	Y	Cervical nodes (April 1923)	8	Yes	...	...	...	Died Feb. 12, 1924 (10 mos.)
3. O. O.	63	M	W	Nasal discharge and cervical nodes (Jan. 1926)	22	Yes	Epistaxis, deafness (June 1926)	...	...	Died June 20, 1928 (40 mos.)
4. M. S.	33	M	B	Cervical nodes (1927)	..	Yes	...	...	...	Died Apr. 12, 1928
5. C. N.	34	M	W	Cervical nodes (1936)	5	Yes	Deafness	VI, July 1937, headache	Yes	Died Oct. 27, 1938 (23 mos.)
6. V. L.*	26	M	W	Cervical nodes (Sept. 1938)	5	Yes	Deafness	Headache	None	Died Nov. 17, 1939 (15 mos.)
7. O. L.*	52	M	W	Cervical nodes (1935)	6	Yes	Deafness	VI, headache	...	Died Aug. 25, 1939 (48 mos.)
8. S. C.*	35	F	W	Cervical nodes (July 1939)	13	Yes	Tinnitus	III, V, VI, VII (R), VI (L), Horner's syndrome	None	Died May 22, 1942 (34 mos.)
9. W. P.*	19	M	B	Tinnitus (Jan. 1940)	2	Yes	Epistaxis and tinnitus	...	...	Died June 25, 1942 (30 mos.)
10. R. A.	15	F	B	Cervical nodes (1940)	11	Yes	Epistaxis, deafness (L)	VI, IX and X	Yes	Died Jan. 1943 (36 mos.)
11. D. L.*	19	M	W	Cervical nodes (Feb. 1940)	1	Yes	...	IX	...	Died Feb. 22, 1941 (12 mos.)
12. M. A.*	59	M	W	Epistaxis (Dec. 1941)	7	Yes	Epistaxis, tinnitus and deafness	IX	Yes	Died Feb. 22, 1941 (13 mos.)
13. V. F.*	18	M	B	Cervical nodes (Dec. 1941)	8	Yes	Deafness, epistaxis and tinnitus	III, V, VI, IX	Yes	Died Mar. 31, 1943 (17 mos.)
14. A. Y.	71	M	W	Epistaxis (May 1942)	6	Yes	Deafness, epistaxis and tinnitus	Headache and ataxia	Yes	Still alive and in good health
15. L. C.*	47	M	Y	Cervical nodes (April 1942)	10	Yes	Epistaxis and tinnitus	...	...	Died Feb. 26, 1943 (10 mos.)
16. C. C.	45	M	W	Pain in back (Oct. 10, 1942)	4	Yes	Nasal obstruction and epistaxis	Deafness	Metastases to spine	Died May 7, 1943 (8 mos.)
17. C. V.	33	M	W	Cervical nodes (June 1942)	9	Yes	Epistaxis and nasal obstruction	VI	None	Still alive and in good health

\* = Autopsy.



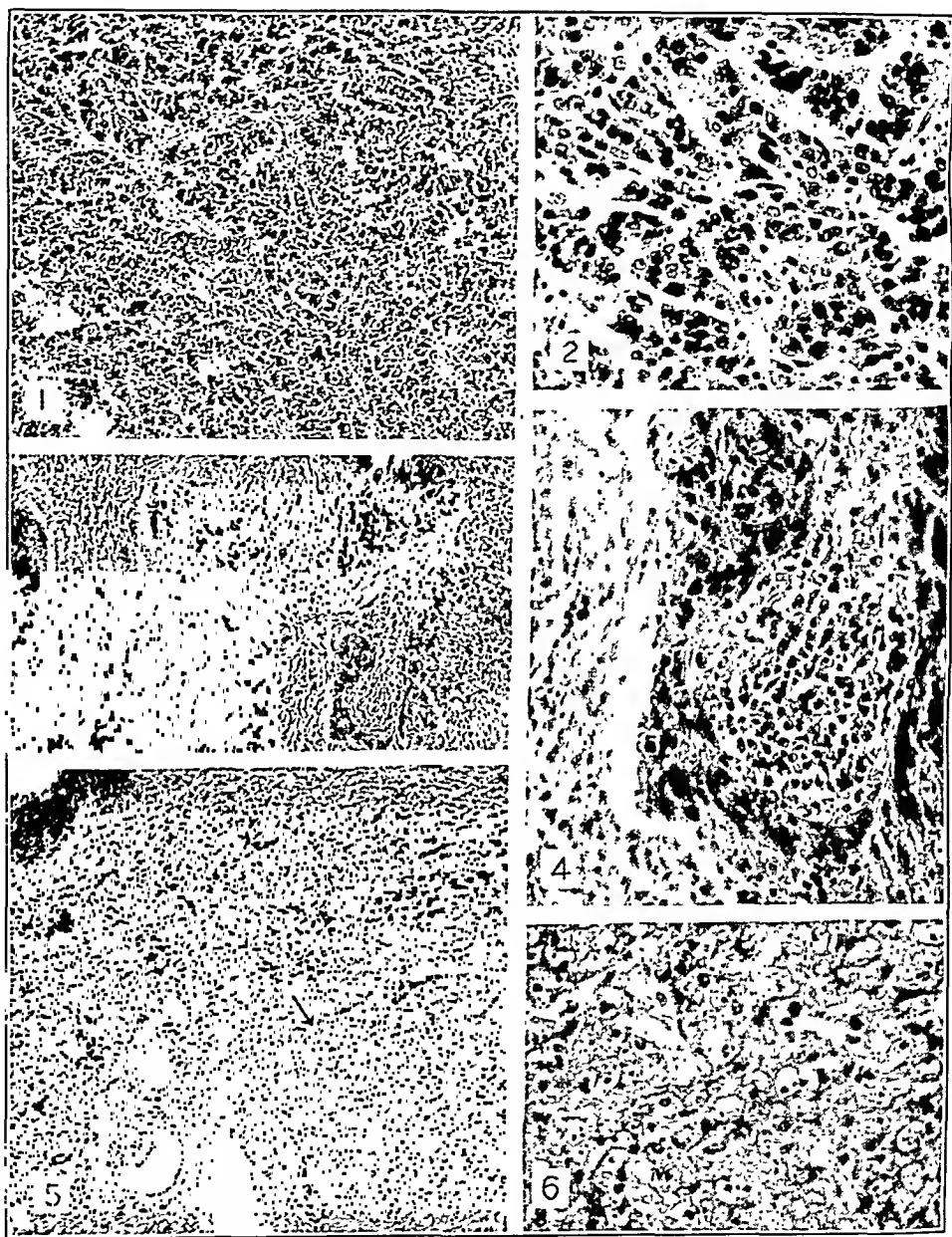


FIG. 1.—Carcinoma of nasopharynx, Group I, as described in text. (Hematoxylin and eosin,  $\times 80$ .)

FIG. 2.—Same as Figure 1 ( $\times 322$ ).

FIG. 3.—Carcinoma of the nasopharynx, Group II, as described in text. (H and E stain,  $\times 80$ .)

FIG. 4.—Same as Figure 3 ( $\times 322$ ).

FIG. 5.—Carcinoma of nasopharynx, Group III, Subtype A, as described in the text. (H and E stain,  $\times 80$ .) The arrows outlined the tumor margin.

FIG. 6.—Same as Figure 5 ( $\times 322$ ).

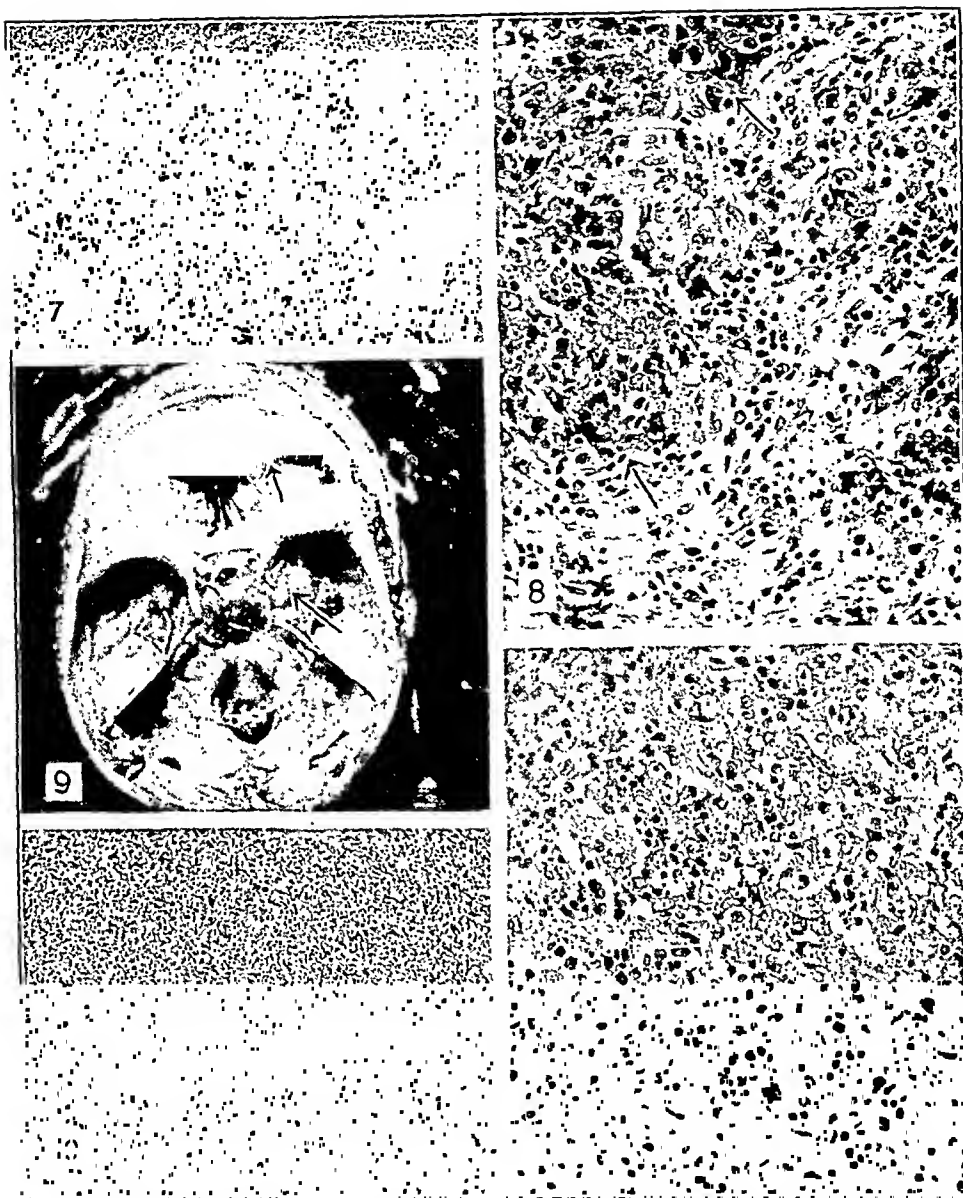


FIG. 7.—Carcinoma of nasopharynx, Group III, Subtype B, as described in the text. (H and E stain,  $\times 80$ .)

FIG. 8.—Same as Figure 7 ( $\times 322$ ). The arrows point to the tumor margin.

FIG. 9.—Case 13, illustrating the path of extension of these tumors up along the internal carotid artery. The posterior arrow points to tumor. The anterior arrow points to tumor nodules in the roof of the right orbit. Subsequent dissection showed the orbit to be filled with tumor which was continuous through the orbital fissure with the portion shown by the posterior arrow. The reason for the frequent involvement of the III, IV, V, VI cranial nerves becomes obvious from this illustration. The right Gasserian ganglion has been forced posteriorly by the tumor.

FIG. 10.—Carcinoma of the nasopharynx, Group III, Subtype C, as described in the text. (H and E stain,  $\times 80$ .)

FIG. 11.—Same as Figure 10 ( $\times 322$ ).

*Subtype B* (3 cases). Instead of showing large blocks of tissue, this tumor features poorly organized, irregular nests and cords of tumor cells creeping out into the nearby stroma. The nuclei of the cells here are of the same general nature. They are considerably smaller than those of Subtype A but are pale-staining with large nucleoli and delicate linin network. In this tumor there is a generous sprinkling of lymphocytes in between the tumor cells in contrast to their relative absence in Subtype A. Cases 7, 9 and 12 are of this type (Figs. 7 and 8).

*Subtype C* (2 cases). This type represents the least organization. Very thin cords of cells weave an entirely irregular pattern. Once again the cells are essentially the same as the above subtypes. The prominent nuclei in the thin cords of cells create the appearance of a diffuse scattering of cells throughout the stroma. Many lymphocytes are seen generally in amongst the tumor cells. Cases 3 and 10 show this structure (Figs. 10 and 11). No doubt, it is Subtype B and C which Schmincke referred to as "lympho-epitheliomas."

The postmortem studies of these cases reveals an interesting tendency for the tumor in Group I to metastasize widely to lungs, liver, and so forth, in addition to local extension into the middle fossa of the skull. The tumors in Group III show a striking tendency to local metastasis to cervical nodes and direct extension up along the internal carotid artery into the middle fossa. The latter cases have not shown general metastases. There is no evidence that these cases have shown symptoms earlier, and that as a consequence came under irradiation therapy earlier. Further study along this line should prove informative.

**Comment.** It should be pointed out that profoundly ill patients can be dramatically relieved temporarily by irradiation. In several of our recent cases patients were rendered symptom-free for weeks or months with complete clinical regression of the primary and also the cervical node enlargement. Subsequent recurrence of the tumor or the metastases has been found to be radioresistant; therefore, once the diagnosis has been established, all patients should be treated to the limit of the skin and physical tolerance even though clinically one can no longer find disease. The practice of irradiating cervical lymph node enlargement without adequate examination of the nasopharynx is to be condemned.

**Summary.** A series of 17 original proven cases of carcinoma of the nasopharynx has been presented with their clinical and pathologic characteristics. The lack of general cognizance of this tumor and therefore its late diagnosis has been emphasized. The clinical syndrome as presented above represents late disease. The radiosensitivity of these tumors leads to marked temporary improvement, hence it is of value even in very advanced cases.

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**"DRUG FEVER" ACCOMPANYING SECOND COURSES OF  
SULFATHIAZOLE, SULFADIAZINE AND SULFAPYRIDINE**

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THE phenomenon of fever occurring as a result of therapy with sulfanilamide was first described by Hageman and Blake,<sup>2</sup> and has since been commonly observed by all clinicians making extensive use of any of the sulfonamide drugs. Such "drug fevers" often appear around the 7th to the 10th day of treatment with a sulfonamide drug, although they may occur at any time after treatment is begun. They are frequently accompanied by dermatitis with or without conjunctivitis. Lyons and Balberor<sup>3</sup> reported that 19 (36%) of 53 patients who received a second course of sulfathiazole experienced fever after failing to develop fever during a first course, whereas the percentage of patients who developed fever during a first course of sulfathiazole which lasted for 7 days or longer was 10.4%.

Several questions occurred to us with regard to the application of these findings: First, will drug fever occur as frequently following two courses of the other sulfonamides as it does following two courses of sulfathiazole? Second, is a second course of a different sulfonamide as likely to cause fever as a second course of the same drug? Third, is this phenomenon related to the length of the first course, to the length of the interval between courses, or to the length of the second course of sulfonamide administration? Further, if a patient does not develop fever from two courses of a sulfonamide is he likely to develop it following later courses? In an attempt to answer these questions, we have analyzed the data on all the patients coming under our observation who have received more than one course of the sulfonamide drugs and who did not develop fever during the first course.

**Results.** As shown in Table 1, among 53 patients who received a second course of sulfathiazole, fever developed during this course in 9 (16.7%). When sulfadiazine\* was given to 68 patients following an earlier course of the same drug 5 (7.4%) developed fever during the second course. Only 22 patients in our series received a second course of sulfapyridine. Two of these (9.1%) developed "drug fever." When the 144 patients receiving a second course of any of these 3 sulfonamides after an initial course of the same drug are considered as a single group, it will be seen that 16 (11.1%) experienced fever during the second course.

The second column, of Table 1 shows those patients who received a course of one of the sulfonamide drugs following an initial course of some

\* Sulfadiazine for this study was supplied by the Lederle Laboratories, Inc.

other sulfonamide. In some instances an interval elapsed between the two courses, and in others the second sulfonamide was given immediately following cessation of administration of the first. Since there was no discernible difference between the proportion of patients developing fever after an interval had elapsed and those in whom the two drugs were given one after the other, we have included them all in the one group. Eighty-nine patients were given sulfathiazole after a previous course of another sulfonamide had not resulted in the production of fever. Five of these patients (5.6%) developed drug fever. One of the 57 patients receiving sulfadiazine and none of the 23 patients receiving sulfapyridine following the administration of a different sulfonamide developed drug fever. When this group is totaled, it is seen that among 169 patients receiving a second sulfonamide after the first failed to produce fever, 6 patients (3.6%) developed drug fever.

TABLE 1.—PATIENTS DEVELOPING FEVER DURING VARIOUS TYPES OF SULFONAMIDE ADMINISTRATION

Previous course of	Same sulfonamide	Another sulfonamide	No previous sulfonamide
<b>Sulfathiazole:</b>			
All cases . . . .	53	89	172
Febrile cases . . . .	9	5	14
% febrile . . . .	16.7	5.6	8.1
<b>Sulfadiazine:</b>			
All cases . . . .	68	57	371
Febrile cases . . . .	5	1	16
% febrile . . . .	7.4	1.8	4.3
<b>Sulfapyridine:</b>			
All cases . . . .	22	23	194
Febrile cases . . . .	2	0	7
% febrile . . . .	9.1	0	3.6
<b>All 3 sulfonamides:</b>			
All cases . . . .	144	169	737
Febrile cases . . . .	16	6	37
% febrile . . . .	11.1	3.6	5

The two groups described above may be compared with those who were observed while receiving their first course of a sulfonamide. Only patients who received the drugs for 6 days or longer, are included in these figures. Sulfathiazole was given to 172 patients among whom 14 (8.1%) developed fever. Sixteen (4.3%) of the 371 patients receiving sulfadiazine experienced fever, as did 7 (3.6%) of the 194 to whom sulfapyridine was administered.

When these results were tested by the chi square method, the figures in the last line where patients treated with the 3 sulfonamides are computed together, are statistically significant. The proportion of patients developing fever upon receiving sulfathiazole after a first course of sulfathiazole is significantly greater than the proportion in patients receiving sulfadiazine following a first course of sulfadiazine. Likewise the proportion of patients developing fever upon receiving sulfathiazole following sulfathiazole is significantly greater than that in patients receiving sulfathiazole following another drug, but the difference between those receiving sulfathiazole following sulfathiazole compared to those receiving a first course of sulfathiazole does not

quite fill the criteria for statistical significance (one-half  $p$  is slightly over 3% whereas it should be 2.5% or less to be considered significant).

It will be noted, however, that even in the instance of those individual drugs (sulfadiazine and sulfapyridine) where the number of cases is not sufficient for the figures to be significant, the trend is in the same direction as the results obtained with the use of sulfathiazole. Furthermore, as has been noted above, the figures for the totals on all 3 drugs are significant.

*Patients Receiving More Than Two Courses.* Three courses of a sulfonamide were administered as follows: sulfathiazole, 3 patients; sulfadiazine, 2 patients; sulfapyridine, 1 patient. Four courses of sulfathiazole were given to 1 patient and four courses of sulfadiazine to 4 patients. One patient was given six courses of sulfadiazine. None of these patients had a febrile reaction attributable to sulfonamides at any time. The longest period covered from the beginning of the first course to the end of the last was 596 days, during which four courses of sulfadiazine were given, separated by three intervals during which no sulfonamides were administered.

*Influence of Length of Courses or of the Interval Between.* In the patients who received two courses of the same sulfonamide, the length of the first course varied from 1 to 35 days (mean 7.1 days) in the sulfathiazole-treated patients; from 1 to 64 days (mean 10.7 days) in the sulfadiazine-treated patients, and from 1 to 31 days (mean 6.8 days) in the patients to whom sulfapyridine was administered. We were unable to find any relationship between the length of the first course and the development of fever during the second course. The length of the interval between courses varied from 1 day to 27 months in the case of sulfathiazole, between 1 day and 19 months in the sulfadiazine-treated patients and between 1 day and 22 months in the sulfapyridine-treated patients. As will be seen from Table 2, febrile reactions occurred after varying intervals, the shortest period of time elapsing between courses being 1 day and the longest 7 months and 6 days. Febrile reactions were no more frequent when the interval was short than when it was long and *vice versa*.

The length of the second course of sulfathiazole varied from 1 to 17 days; of sulfadiazine from 1 to 79 days, and, in the case of sulfapyridine, from 1 to 12 days. Again, we were unable to find any relationship between the length of the second course and the incidence of drug fever. When a febrile reaction did supervene, it began anywhere from the 1st to the 7th day of the second course of the drug. When the total length of time covered by the first course of the sulfonamide, plus the interval, plus the second course was considered, it bore no relation to the number of febrile reactions.

Table 2 also shows that a drug rash was present in addition to the febrile reactions in 6 of the 9 patients with fever following a second course of sulfathiazole; in 3 of the 5 patients with fever following a second course of sulfadiazine and in both of the patients experiencing fever following a second course of sulfapyridine. Conjunctivitis was present in addition to dermatitis in 1 patient each receiving sulfathiazole and sulfadiazine.

TABLE 2.—PATIENTS WITH FEBRILE REACTION TO SECOND COURSE OF SULFONAMIDES

Case No.	Duration of first course (days)	Interval between courses (days)	Day of second course when fever began	Dermatitis or conjunctivitis
<i>Sulfathiazole Following Sulfathiazole</i>				
1 . . . . .	6	2	5	No
2 . . . . .	20	3	1	D
3 . . . . .	7	14	4	D
4 . . . . .	1	3	7	D
5 . . . . .	2	8	3	D
6 . . . . .	1	28	2	No
7 . . . . .	8	24	2	No
8 . . . . .	4	4	2	D and C
9 . . . . .	4	21	5	D
<i>Sulfadiazine Following Sulfadiazine</i>				
10 . . . . .	10	6	5	D
11 . . . . .	21	7 mos. and 6 days	1	No
12 . . . . .	13	6	1	D
13 . . . . .	4	1	1	D and C
14 . . . . .	20	6	1	No
<i>Sulfapyridine Following Sulfapyridine</i>				
15 . . . . .	9	2	10	D
16 . . . . .	20	5	2	D

**Discussion.** We have been able to confirm the findings of Lyons and Balberor<sup>3</sup> that fever follows the administration of a second course of sulfathiazole more frequently than it occurs during a single course and to show that this result is also obtained after therapy with other sulfonamides, that is, sulfadiazine and sulfapyridine. While the percentage of febrile reactions in our series following a second course of sulfathiazole (17.1%) was not as high as that found by Lyons and Balberor<sup>3</sup> (36%), this may have been due to a difference in the dosages used in the second course. The former authors gave 4 gm. of sulfathiazole followed by 1 gm. every 4 hours. In approximately one-half of our cases this dose was given, while in the others, a dose of 2 gm. initially and 0.5 gm. every 4 hours was used. Febrile reactions tended to occur more frequently in the patients who received the higher doses.

Fink and Wilson<sup>1</sup> found that among 86 children given sulfathiazole, 3 developed febrile reactions; and that among 91 who received sulfadiazine, 4 experienced febrile reactions. It is possible that the fact that the subjects used in this investigation were all children may have accounted for the low incidence of drug fever, since it has been the experience of many observers that reactions from the sulfonamides in general are less frequent in children than in adults. Unfortunately, the authors did not state for comparison the number of febrile reactions which occurred in children under their observation receiving only one course of the sulfonamides. On the other hand, the low incidence of febrile reactions may have been due to the use of low dosages necessitated by the fact that the subjects were children.

If a second course of a sulfonamide is given to an adult after an interval of time has elapsed since the administration of a course of the same sulfonamide, a febrile reaction is likely to occur, especially if a full dose of the drugs is used. On the other hand, as the second line

of Table 1 shows, if another sulfonamide is given for the second course, instead of the same sulfonamide, febrile reactions are not any more frequent than during a single course of a sulfonamide and may possibly occur less often. The practical application of this is obvious; if a second course of a sulfonamide is needed and another drug is available which is as potent as the first one, the second drug should be used for the succeeding course. Apparently, the length of time covered by the first or second courses or of the interval between has no effect upon the tendency for febrile reactions to develop, except that none of the febrile reactions developed earlier than the 10th day following the commencement of the first course of the drug, with the exception of 1 case in which the fever began after 4 days of sulfadiazine therapy, a 1-day interval during which no drug was given and less than 1 day of subsequent sulfadiazine administration. By contrast, none of the patients who received a different sulfonamide during the second course developed a febrile reaction earlier than the 3rd day of administration of the second sulfonamide.

Since none of our 12 patients who were given from 3 to 6 courses of a sulfonamide developed febrile reactions during any course, it is possible that patients who are going to develop drug fever will do so during the first or second course or not at all. A larger group of patients who have received multiple courses will be needed to prove this point, however.

**Summary and Conclusions.** 1. Among 144 patients who received a second course of sulfathiazole, sulfadiazine or sulfapyridine after a varying interval of time had intervened since the first course of the same sulfonamide, 16 (11.1%) developed drug fever. Eleven of these patients developed concomitant dermatitis, which in 2 instances was associated with conjunctivitis.

2. Among 169 patients who received a second course of sulfathiazole, sulfadiazine or sulfapyridine following a first course of another sulfonamide, 6 (3.6%) developed drug fever.

3. Among 737 patients who were observed during a single course of therapy with sulfathiazole, sulfadiazine or sulfapyridine, 37 (5%) developed febrile reactions.

4. Febrile reactions were more frequent following a second course of sulfathiazole as well as during a single course of sulfathiazole than was the case with sulfadiazine or sulfapyridine.

5. Three to 6 courses of the same sulfonamide were given to 12 patients, none of whom developed febrile reactions.

6. It is concluded that, when a second course of a sulfonamide must be given to a patient, regardless of the interval following the first course, another sulfonamide drug should be given.

We wish to thank Dr. Lewis K. Sweet for his coöperation in this study, and Miss Louise Mause and Miss Ruth Mayer for technical assistance.

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## INSULIN RESISTANCE

## THE RÔLE OF IMMUNITY IN ITS PRODUCTION

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It is well known that insulin is a poor antibody producer. Barral and Roux,<sup>3</sup> Lewis<sup>18</sup> and Bernstein, Kirsner and Turner<sup>5</sup> were able to demonstrate sensitization of guinea pigs by means of the Schultz-Dale technique or by anaphylactic reactions. More recently Wasserman, Broh-Kahn and Mirsky<sup>25</sup> have demonstrated antibodies in rabbits injected with insulin by means of the complement-fixation technique. Other investigators have failed to produce antibodies to insulin. In my own experiments rabbits and guinea pigs receiving insulin subcutaneously and intraperitoneally failed to develop skin hypersensitivity or circulating antibodies as measured by the precipitin test, nor did their serum protect a normal animal against a minimal lethal dose of insulin.

The fact that insulin is a poor antigen is rather fortunate for diabetics. Many diabetic patients receiving insulin for the first time develop local reactions. Such reactions usually disappear after a few injections, although they may persist for a long time in an occasional case. In either event such patients respond to insulin in the usual fashion.

Some patients become hypersensitive to insulin, developing generalized as well as local reactions. Some of these patients have been reported to have positive skin tests to more than one type of insulin,<sup>2,4,6,9,10,24</sup> passive-transfer antibodies,<sup>7,22,24</sup> and precipitins.<sup>24</sup> They may or may not respond to insulin in the usual fashion. Only an occasional patient becomes resistant or refractory and requires tremendous doses to control the diabetes. Several such patients have been reported and in a few instances immunologic studies have been made. The patient described by Glassberg, Somogyi and Taussig<sup>12</sup> developed local and urticarial reactions first, and later, insulin resistance, with disappearance of skin reactions. This patient's serum did not contain precipitins. The cases of insulin resistance reported by Wiener,<sup>28</sup> Martin, Martin, Lyster and Strouse<sup>21</sup> and Glass, Spingarn and Pollack<sup>11</sup> showed no passive-transfer or other types of antibodies. On the other hand, the patient of Karr, Kreidler, Seull and Petty<sup>15</sup> showed positive skin tests to all types of insulin, passive-transfer antibodies and precipitins for insulin. All of these reports agree that the patients' serum does not antagonize the hypoglycemic effect of insulin in animals. On the other hand, Cannon and co-workers,<sup>8</sup> Lowell,<sup>19</sup> and the author<sup>16</sup> have obtained evidence of the opposite sort, which will be discussed more fully below. It is true that Glen and Eaton<sup>13</sup> and Wayburn and Beekh<sup>27</sup> have also demonstrated an insulin-antagonizing effect of the serum from such patients,

but they attributed it to an anterior pituitary diabetogenic factor. Similarly Marble, Fernald and Smith<sup>20</sup> were inclined to attribute the delayed anti-insulin effect of the serum from 2 insulin-resistant patients to the action of a pituitary factor.

**Material and Method.** The blood from 6 patients with insulin resistance was examined for the presence of insulin antibodies. The ring precipitin method and the Prausnitz-Kustner test were largely employed. In performing the precipitin test the antiserum was absorbed with bovine and pig serum previous to testing, and dilutions of the antigen, starting with 0.5 or 1% solution of crystalline zinc insulin,\* were layered over the sera. In some instances amorphous insulin from different sources was substituted, without affecting the results. In the Prausnitz-Kustner test, 2 units of crystalline zinc insulin were used to test for the presence of passive-transfer antibodies. In addition the sera from several patients suspected of being resistant to insulin, and from patients showing transient or persistent local reactions to insulin were examined by these methods. In some instances the attempt was made to determine if the sera from resistant cases would protect guinea pigs against a lethal dose of insulin. In 1 case, studied more extensively than the others, the "insulin tolerance test" was determined and compared with such a test in the usual diabetic case.

TABLE 1.—IMMUNE REACTIONS OF SERUM FROM INSULIN-RESISTANT PATIENTS AND THEIR INSULIN REQUIREMENT

Case No.	Antiserum incubated with bovine and pig serum	Dilutions of crystalline zinc insulin 0.5%						Passive transfer test	Daily insulin dosage (units)	
		1	4	16	64	256	1000			
1	E.St.C. (a)	+++	+++	++	++	++	+	+++	450	1/9/41
	E.St.C. (b)	++	+	±	—	—	—	+++	100	2/25/42
	E.St.C. (c)	++	++	++	+	±	—	+++	200	3/21/43
2	Fr.P.	—	—	—	—	—	—	Neg.	50	Neg. precip. test with dilutions of 1% insulin
3	A.LeV.	—	—	—	—	—	—	Neg.	63	"
4	E.P. (a)	+	+	±	—	—	—	++	200	
	E.P. (b)	—	—	—	—	—	—	Neg.	46	"
5	H.T.	—	—	—	—	—	—	+	160	"
6	A.M.	—	—	—	—	—	—	+++	200+	"

Heating serum for 1 hour at 56° C. did not affect the results.

**Results.** The results of the precipitin tests are summarized in Table 1. This table deals only with data obtained on 6 patients who showed evidence of insulin resistance. The sera from several normals and from several diabetic patients were tested, including the sera from 2 patients in whom insulin injections caused persistent local reactions. They all failed to show circulating antibodies to insulin or passive-transfer antibodies. The sera from some of the patients receiving insulin did show a small titer of antibodies to bovine or pig serum (1:2 to 1:10 dilution of antigen).

**CASE 1.**—This patient, E. St. C., came under observation at this hospital in October, 1940. She had had rheumatoid arthritis for 13 years and diabetes for 6 years. She had been treated from the beginning of her diabetes by members of the Joslin Clinic. They discovered that about 2 months after the beginning of insulin treatment her requirement for insulin rose to 400 to

\* I am indebted to Dr. F. B. Peck of Eli Lilly & Company for a supply of crystalline zinc insulin.

500 units daily. At times as much as 700 units daily were used in an attempt to control the glycosuria; she was resistant to any type of insulin, including crystalline insulin. In addition she had severe local reactions, resulting in subcutaneous nodules of inflammation and necrosis at the sites of injection. During her 4-month stay at the Massachusetts General Hospital the dosage of insulin varied between 150 and 450 units daily. This resistant state was

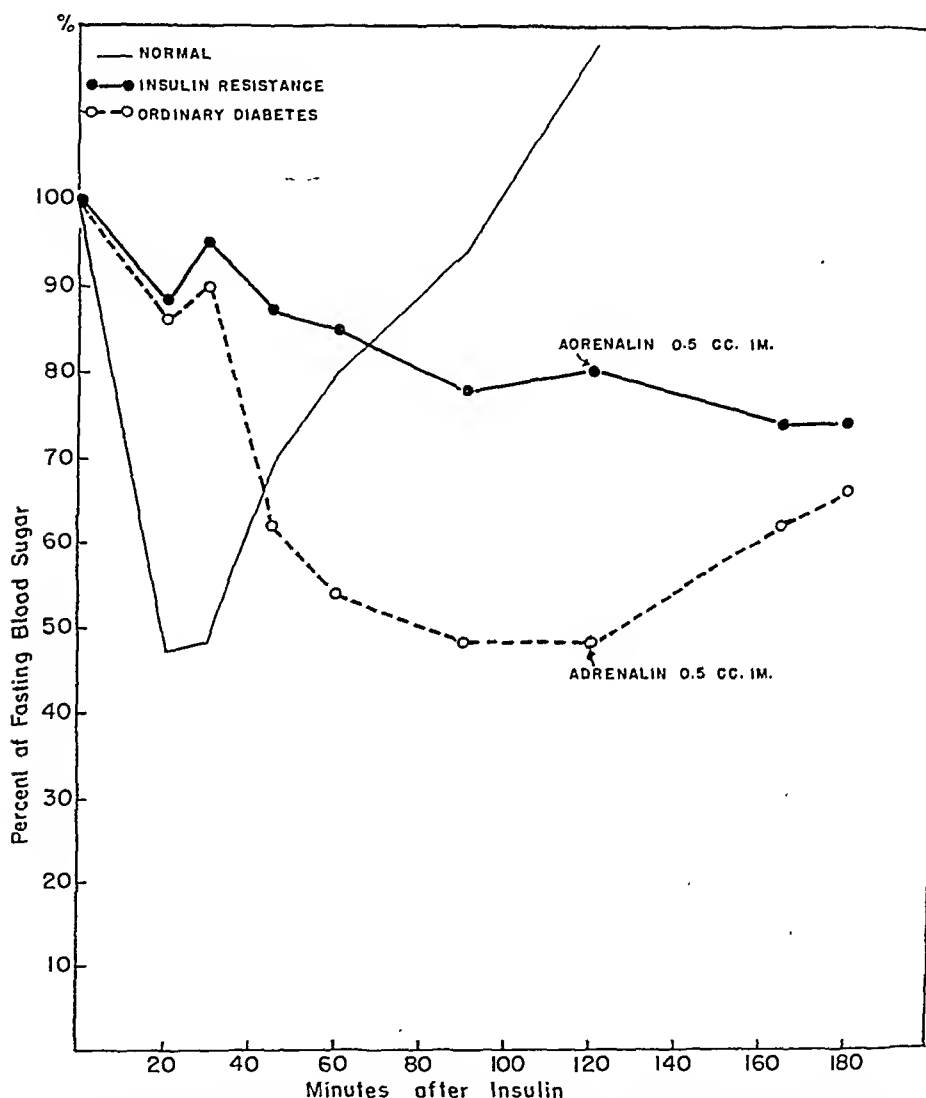


FIG. 1.—The insulin tolerance test of a patient with insulin resistance (E. St. C.) compared with that of a patient with ordinary diabetes. The "normal" insulin tolerance is charted for reference.

verified by the fact that 10 units of insulin intravenously failed to produce the expected drop in blood sugar. As shown graphically in Fig. 1, the blood sugar of the usual diabetic falls rapidly to 47% of the fasting level in 90 minutes. In other words, the ordinary diabetic is "insulin-sensitive." On the other hand, the blood sugar of this patient dropped very little, reaching 78% of the fasting level in 90 minutes.

The serum of this patient contained circulating antibodies to insulin to a dilution of antigen of 1:200,000 (Table 1) and also gave a positive Praussnitz-Kustner test. Several months previously, Dr. Paul Cannon<sup>8</sup> also had found agglutinins for insulin and passive-transfer antibodies in her serum.

For several months after she left the hospital the insulin requirement was 700 units daily, but by February 1942, when another blood sample was obtained, her requirement had dropped to 100 units daily. At this time the precipitin reaction was positive only up to a dilution of 1:800. The serum still gave a positive Praussnitz-Kustner test up to a dilution of serum of 1:90. At the present, the precipitin test is positive up to a dilution of 1:12,000, the passive-transfer test is positive up to 1:90, and the insulin requirement is 200 units daily.

An attempt was made to determine the physiologic activity of this serum. Theoretically such a serum should protect an animal against a lethal dose of insulin; or, if injected into an animal without insulin, it should cause a rise in blood sugar by antagonizing the animal's own insulin. The results of such experiments with this serum were equivocal, due, perhaps, to inadequate amounts of serum used.

The results in 5 other cases are recorded in Table 1. The serum of 2 patients (Fr. P. and A. LeV.) was made available through the kindness of Drs. Root and Marble. Both of these patients had gone through a period of insulin refractoriness for about a year. Their requirements ranged up to 2200 units daily, which they received without interruption. Unfortunately their serum was not examined during this phase for precipitins or passive-transfer antibodies; but Marble, Fernald and Smith<sup>20</sup> did find on 1 occasion an anti-insulin substance in the serum, which had a delayed effect. Later, when the requirement for insulin had returned to a level nearer normal (50 to 60 units daily), their serum contained neither circulating antibodies to insulin, nor passive-transfer antibodies.

The 4th patient (E. P.) was first seen on the wards of the Massachusetts General Hospital where his insulin requirement fluctuated between 100 to 140 units. At this time circulating antibodies against insulin were not demonstrable in the blood and it was felt that the high requirement for insulin was due to infection. However, 3 months later, when his requirement for insulin rose to 200 units daily, a blood sample obtained for us by Dr. Root gave a positive precipitin test up to a dilution of 1:800, and a moderately positive passive-transfer test. In addition 1 cc. of serum injected into each of 2 guinea pigs delayed the effect of a lethal dose of insulin in one, and neutralized it completely in the other. At present (a year later), his blood contains neither precipitins nor passive-transfer antibodies, and his insulin requirement is down to 46 units daily.

The serum of the 5th case (H. T.) was obtained from a patient of Dr. D. Hurwitz. No precipitins were found in this serum but a slight concentration of passive-transfer antibodies was demonstrable. The insulin requirement of this patient was only 160 units, indicating a rather mild degree of insulin resistance. Several months later the insulin requirement dropped to 60 units, but blood was not available for study.

The last patient (A. M.) displayed both insulin resistance and insulin sensitivity, and has been reported on by Dr. F. C. Lowell,<sup>19</sup> who was

kind enough to send us blood specimens for study. Intravenous insulin had no effect upon the blood sugar of this patient. Samples of blood taken during the administration of insulin and 1 month after its omission failed to show precipitins or agglutinins. One cc. of serum injected intravenously into a rabbit had no effect upon the blood sugar level of the rabbit over a period of 4 hours. On the other hand, the Praussnitz-Kustner test was strongly positive, the reaction lasting over 1 hour. Moreover, 1 cc. of serum injected intraperitoneally into each of 3 guinea pigs either prevented or delayed the hypoglycemia produced by 0.2 mg. of crystalline zinc insulin. These experiments along with those in Case 4, although few in number, confirm the antihormonic effect of such serum.

**Discussion.** The clinical fact that insulin, administered subcutaneously or intravenously, fails to affect the blood sugar in an insulin-resistant diabetic, in the presence of various types of antibodies to insulin, suggests immediately that the antibodies to insulin are the cause of the resistant state. These antibodies, either circulating or fixed, are able to neutralize injected insulin so that the requirement for insulin to control the diabetes is considerably increased. In other words, antibodies to insulin are antihormonic. The demonstration of substances in the serum of such a patient, which protect animals against the hypoglycemic effects of insulin, is strongly confirmatory of this viewpoint. Various types of antibodies are present, but not all types are demonstrable in any given case. For example, the serum of Case 1 failed to show biologic antagonism against insulin in spite of a high concentration of precipitins and passive-transfer antibodies, whereas the serum of Case 6 contained no precipitins but antagonized the action of insulin and gave a strong Praussnitz-Kustner test.

The data obtained in Case 1 strongly suggest that the degree of insulin resistance parallels the concentration of antibodies. This is also suggested by the results in Case 4. Here the precipitin and passive-transfer tests turned positive under observation as the degree of insulin resistance increased, and later became negative again as the insulin requirement dropped. In addition, Case 5 showed a negative precipitin test and only a mild passive-transfer test in the presence of a mild degree of insulin resistance.

The data in Cases 2, 3 and 4 suggest that the return of normal insulin sensitivity is dependent upon the disappearance of immune bodies. Similar fluctuations in antibodies have been observed in humans, such as the appearance of antibodies to cow's milk protein, egg white and other proteins when infants are first exposed to these foods and the subsequent disappearance of the antibodies, in spite of continued ingestion of such foods.<sup>1</sup> Likewise, I have observed the disappearance of antibodies to thyroglobulin in rabbits repeatedly injected with thyroglobulin. It would seem that repeated injections of insulin, rather than omission of insulin, offers a mode of therapy in bringing about desensitization and cure of insulin resistance. Theoretically, the worst thing one could do in such cases is to give insulin

at infrequent intervals. Such a procedure should enhance rather than diminish antibody production.

It has been reported by various investigators and confirmed by the results reported above, that the serum of insulin-resistant patients reacts almost alike with insulins from different animal sources, in spite of the fact that the resistant state may have been induced by a variety of insulin preparations. Moreover, such patients respond as poorly to one type of insulin as to another, including protamine insulin and crystalline zinc insulin. In other words, antibodies to insulin, in common with antibodies to other hormones, are hormone-specific rather than species-specific? In the case of antibodies to thyroglobulin, hormone specificity has been described by Hektoen and Schulhof<sup>14</sup> and by myself;<sup>17</sup> in the case of antibodies to the anterior pituitary this type of specificity has been described by various investigators.<sup>23</sup> Experimentally, the immunologic identity of insulins from various species has been shown by Lewis,<sup>18</sup> who used the Schultz-Dale technique, and by Wasserman and Mirsky,<sup>26</sup> who used the complement-fixation reaction.

It must be emphasized that hormone-specificity is only relative. The reaction of the antiserum may be greater with the hormones derived from some species than with those derived from others. It is weakest with the hormone derived from the donor species. For example, antiserum obtained by injecting rabbits with human thyroglobulin is 100 to 1000 times as potent when tested with human thyroglobulin as when tested with rabbit thyroglobulin. By analogy, the serum from insulin-resistant patients should antagonize human insulin much less than insulins derived from other species. This fact may explain some of the results reported by Lowell.<sup>19</sup> In his patient with insulin resistance, 30 units of human insulin caused a marked drop in blood sugar, whereas 30 units of crystalline insulin (animal) failed to do this. This is as expected on the basis of the analogy with thyroglobulin just mentioned. The comparison should be between 30 units of human insulin *versus* 3000 or more units of crystalline insulin (animal). Furthermore, his inability to demonstrate "protective antibodies" to human insulin in the presence of such antibodies to crystalline zinc insulin is also as expected. Any biologic protection test is undoubtedly too crude to detect slight amounts of antibodies to an antigen derived from the same species as the anti-serum is derived.

It is interesting to speculate as to what happens to the patients who survive the period of insulin resistance. At present, Patient 2 is controlled by 50 units of insulin daily, Patient 3 by 63 units, Patient 4 by 46 units, and Patient 5 by 60 units. The first patient is still resistant, and there are no further data on the sixth. The patient reported by Wiener<sup>28</sup> was controlled by 50 units of insulin 3 years after the episode of insulin resistance, and the patient of Glassberg, Somogyi and Taussig<sup>12</sup> took 50 units for 14 years after recovery from the insulin-resistant state.\* All of those patients were mildly diabetic before the phase of insulin resistance, requiring only small amounts of insulin to

\* End-results on last 2 cases cited by Glass, Spingarn and Pollack.<sup>11</sup>

remain sugar-free. Only the patient reported by Glass, Spingarn and Pollack<sup>11</sup> remained a mild diabetic after recovery from insulin resistance. In view of the findings reported by the author<sup>17</sup> that animals immunized by thyroglobulin become permanently myxedematous, one may speculate that patients immunized by insulin (insulin-resistance) may completely lose the capacity to produce insulin and thereby become total diabetics. If this is true, then such complete diabetics apparently require 50 to 60 units of insulin daily (Table 1). Stated another way, non-diabetic persons produce about 50 to 60 units of insulin daily.

**Conclusions.** 1. Antibodies to insulin appear to be antihormonic.

2. Insulin resistance is dependent upon the appearance and concentration in the body of antibodies to insulin.

3. The return of normal insulin sensitivity is dependent upon the disappearance of the immune response.

4. Repeated administration of insulin, rather than its omission, offers a means for overcoming insulin resistance.

5. Antibodies to insulin simulate antibodies to other hormones by being hormone-specific rather than species-specific.

6. Patients recovering from insulin resistance usually become severe diabetics, requiring 50 to 60 units of insulin daily. It is suggested that this represents the level of complete diabetes.

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## THE USE OF GLYCOL VAPORS FOR BACTERIAL CONTROL IN LARGE SPACES\*

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THE bactericidal effect of germicidal mists and vapors on air-suspended microorganisms has now been well established and demonstrated by many investigators.<sup>1,4,7,11</sup> As a result of recent studies it appears that propylene or triethylene vapor offers certain advantages over the use of aerosols and mists previously advocated.<sup>3,8,9,10</sup> The glycols in vapor form are effective in such low concentrations as to be imperceptible in the treated space; they are odorless and non-toxic in the recommended quantities, and the amount necessary to produce air sterilization is so small that the cost is minimal.

Most studies up to this time have been carried out in small test chambers with little accurate data available on large spaces. The chief obstacles to more careful study and application of glycol control of airborne infections in a practical way have been the absence of effective and simple methods of introducing and maintaining adequate concentrations in the desired space and the knowledge of distribution of the vapor in the space. This study reports findings on the distribution. Further information on the apparatus for glycol introduction will be published shortly.

**Materials and Methods.** *Location of Tests.* All our studies were carried out in a special air-conditioned room (Fig. 1) which was constructed for ventilating research at Northwestern Technological Institute. This room, 38 feet long, 17 feet wide, and 16 feet high, has a volume of approximately 10,000 cu. ft. The walls, ceiling, and floor are cork construction for insulation, and the doors and windows are equipped with refrigerator type doors. For these experiments the ducts leading to and from the air-conditioning apparatus were sealed off.

*Glycol Sampling.* The glycol determinations were made on 2 liter samples of room air when propylene glycol was analyzed and on a 1 cu. ft. sample of room air when triethylene glycol was used. For the propylene this quantity was obtained by means of a pump displacing 2 liters of water in 10 minutes from one container to another, the air to be analyzed being bubbled through 10 cc. of distilled water with the aid of a sintered glass disperser. When triethylene glycol was recovered a gas flow-meter replaced the water bottles and 1 cu. ft. of air taken over a period of 10 minutes. Samples were obtained at floor level and at 5 and 10 foot levels at opposite ends of the room and at the center walls.

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The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Northwestern University.



Analyses were made by the methods described by Puck and Wise, using ceric sulfate for the propylene and potassium dichromate for the triethylene.<sup>6</sup> They were recorded as milligrams of glycol per liter of air.

*Introduction of Vapor.* Several methods for the production of glycol vapor were used in this study. An air scrubber was constructed, in which room air was blown through a spray of liquid glycol. The droplets were eliminated by a series of baffles and filters, permitting only the vaporized glycol to escape

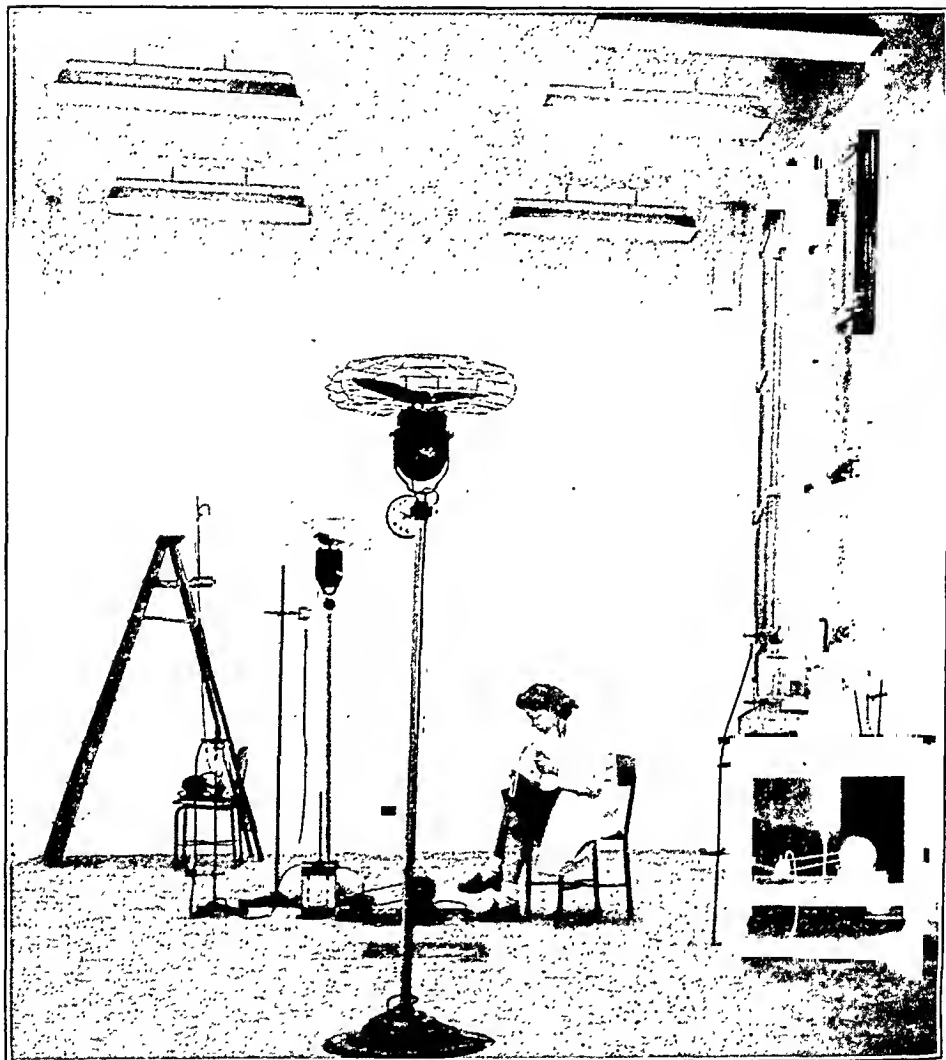


FIG. 1.—Test-room showing sampling apparatus, atomizer and circulating fans.

with the carrier air. This proved to be a very satisfactory apparatus. Another method consisted of blowing room air over the surface of heated glycol. Finally, glycol-water solutions of definite concentrations were heated to their boiling point and thus vaporized into the room.\*

\* A detailed discussion of the construction and operation of these devices is soon to be published. All methods could be controlled so that the quantity and rate of emission of glycol vapor were regulated within close limits. Both the air scrubber and boiling devices are satisfactory for large scale installation.

*Means of Air Agitation.* Glycol samples were taken when there was no air movement in the room and when air was agitated to varying degrees by means of circulating fans (Fig. 1).<sup>\*</sup> The speed of the fans could be regulated by a variable transformer and the direction of air flow controlled by adjusting the angle of the fan-head. Agitation was also produced by the 100 cu. ft. per minute blower incorporated in the air scrubber.

*Bacterial Samples and Counts.* Test organisms used were *Staphylococcus albus* and a guinea pig strain of hemolytic streptococcus (*Streptococcus C*). The suspensions of organisms were prepared by concentrating 50 cc. of an 18 hour culture of the desired bacteria and resuspending the organisms in saline to a standardized concentration, 1 cc. of the suspension containing 1 billion organisms. This was then centrifuged, the supernatant fluid decanted, and the organisms again suspended to their previous volume with filtered saliva. Saliva was used as the diluent since it has been noted that organisms suspended in this material remain in the air much longer than those introduced in broth or saline and we wished to simulate field conditions as closely as possible. The suspensions were sprayed by means of a modified Graeser atomizer<sup>2</sup> which produced an extremely fine mist.

Bacterial samples were obtained by the use of the apparatus described by Moulton;<sup>5</sup> 1 cu. ft. of air was drawn through 10 cc. of broth in the reservoir and 0.5 and 1 cc. samples were plated. Colony counts were made in the usual manner and recorded as numbers of organisms per cubic foot of air. Samples were taken in the same positions as those for glycol analysis, that is, at floor, 5-foot and 10-foot levels at opposite ends and the center of the room.

A test was run as follows: the room was first thoroughly aired out by opening the windows and doors and turning on the two large circulating fans. It was then made air-tight and 10 cc. of the bacterial suspensions were sprayed directly under the two circulating fans revolving at medium speed (1350 r.p.m.) at the two opposite sides of the room. Simultaneous samples were taken immediately after the cessation of the spray and at intervals of 15 minutes for a total period of 2 hours. The doors and windows were reopened, the fans speeded up and the room allowed to air for 1 hour. The room was again sealed, the glycol vapor introduced, and 10 cc. of the same bacterial suspension used for the control test was sprayed under the fans. Simultaneous samples of both glycol and bacteria were taken as described.

*Control of Humidity.* Since small quantities of glycol may collect on the hairs or fine strands of the usual type of humidistat, all observations on humidity were taken by means of wet and dry bulb thermometers. Although the scrubber and boiling apparatus were able to maintain humidity fairly well it was sometimes necessary to introduce additional water vapor into our room, since it has been observed that in order to obtain optimum bactericidal effect from the glycol vapors a humidity of 35 to 40% is desirable. The additional water vapor was added either by heating a container of water or by introducing steam in small quantities.

*Data. Distribution Studies.* Our first object was to determine if it were possible to build up an adequate glycol concentration in a space of 10,000 cu. ft. and maintain it for long periods. This can readily be done and may be produced by any of the methods described, taking 30 minutes to 1 hour depending on the apparatus used. However, the necessity of maintaining adequate humidity causes certain limitations of methods of introduction.

An adequate glycol concentration may be attained theoretically in a space with no air agitation by allowing the vapor to diffuse slowly throughout the room. However, it would appear from repeated observations that some means of air agitation is necessary to produce uni-

<sup>\*</sup> We wish to acknowledge the cooperation of the Reynolds Electric Company, Chicago, Ill., in supplying us with these fans.

form and rapid distribution. This is particularly true if the space is irregular in shape.

TABLE 1.—RATE OF FALL OF PROPYLENE GLYCOL IN TEST-ROOM

Time	Glycol concentration in room air (mg. per liter)		
	At floor level	At 5 feet	At 10 feet
Generator off . . . . .	0.195	0.202	0.269
2 hours later . . . . .	0.196	0.149	0.145
4 " " . . . . .	0.148	0.110	0.110
6 " " . . . . .	0.132	0.106	0.105
24 " " . . . . .	0.048	0.048	0.037
26 " " . . . . .	0.063	0.052	0.052
28 " " . . . . .	0.032	0.040	0.028
30 " " . . . . .	0.036	0.017	0.026

The rate of fall in concentration of glycol in the air was determined for our test chamber, and a typical experiment is shown in Table 1. These values were obtained in the following manner: glycol vapor was introduced into the room and samples were taken until a satisfactory concentration of glycol in the air was reached. The glycol "generator" was then turned off, the room sealed, and air samples taken at the intervals shown in the table. The concentration held up quite well as might be expected in this space. It was of interest also to note that with very gentle air agitation there was no evidence of stratification of glycol vapor in our room (Table 2). The samples taken at floor level, at 5 feet, and at 10 feet were all of equal magnitude. We should also like to point out that even though the space was relatively air-tight it was necessary to introduce a glycol concentration from 50 to 100% greater than the ultimate concentration desired in the room to compensate for exfiltration of air.

TABLE 2.—DISTRIBUTION AND CONCENTRATION OF PROPYLENE GLYCOL IN TEST-ROOM

Time	Glycol concentration in room air (mg. per liter)			Mg. glycol per liter in air delivered from generator
	At floor level	At 5 feet	At 10 feet	
Glycol generator operating for				
1 hour . . . . .	0.090	0.083	0.087	0.229
1 hour later . . . . .	0.127	0.138	0.107	0.312
2 hours later . . . . .	0.125	0.182	0.125	0.307
3 " " . . . . .	0.207	0.164	0.185	0.326

Although the figures recorded on these protocols are for propylene glycol, repeated tests using triethylene glycol showed no difference in the behavior of this vapor in the atmosphere.

*Bacterial Studies.* As a further "check" of the distribution, tests on the bactericidal action of the glycol vapor were made. We were interested in obtaining the immediate killing effect rather than death after prolonged periods of exposure. It was found that whereas in small chamber experiments immediate killing occurred with concentrations of 0.02 mg. of propylene glycol per liter, a concentration of 0.2 mg. per liter was necessary in the large test-room. When triethylene glycol was used an immediate and striking effect was produced in our space

with a concentration of 0.005 mg. per liter.\* The following 2 protocols (Tables 3 and 4) illustrate our findings. Photographs of these data are shown in Figures 2 and 3.

TABLE 3.—EFFECT OF PROPYLENE GLYCOL VAPOR ON *STREPTOCOCCUS C*

Vapor concentration	Humidity (%)	Time interval of samples (after spraying)	No. colonies per cu. ft.	
			Control	Test
0.2 mg. per liter	42	Immediately after	4166	0
	42	15 minutes later	3120	0
	42	30 " "	1760	0
	42	60 " "	442	0

It was observed that in order to produce this effect with either glycol a humidity of about 40% was necessary. When the humidity was lower or when the concentration of glycol in the air was lower, definite reduction of bacterial counts could be produced with no recovery of viable organisms after periods of 30 minutes to 1 hour after exposure. However, for the purpose of adequate and preliminary wide scale testing we felt that the immediate killing effect should be sought. This is perhaps a higher concentration than might ultimately be desired.

TABLE 4.—EFFECT OF PROPYLENE GLYCOL VAPOR ON *Staphylococcus albus*

Vapor concentration	Humidity (%)	Time interval of samples (after spraying)	No. colonies per cu. ft.	
			Control	Test
0.2 mg. per liter	41	Immediately after	9750	0
	41	15 minutes later	9360	0
	41	30 " "	9260	0
	41	60 " "	3220	0

**Discussion.** Since we have observed that there is ready diffusion of vapor about the space to be treated it would theoretically be possible to introduce glycol vapor into a space and allow it to distribute itself spontaneously. However, from a practical standpoint this would never be adequate to insure uniform distribution due to air currents in and out of the space and leakage of air out of the space. Thus it is necessary to have some means of air agitation. This may be produced either by the presence of circulating fans or by a simple duct system. The size, speed, number, and position of the circulating fans must be determined by the physical characteristics of the space to be treated. For portable and emergency use this type of fan is the simplest and easiest to use.

When a more permanent installation is feasible a duct system may be constructed. Since glycol is a relatively inert substance the construction of these ducts may be simple. They may be made of almost any structural material ranging from fiber board (masonite) to metals. An example of this type of duct layout is shown in Figure 4. Observe that a duct leading outside is installed. The quantity of outside "fresh air" introduced may be regulated by a damper. Our plans have contemplated 10 cu. ft. per minute for each individual in the space but

\* Misunderstanding and confusion have arisen from the expression of glycol concentration in "parts per million" used in previous publications. For this reason we suggest the use of weight per volume (milligrams per liter of air). This may be converted to grams per million cc. of air if one so desires, for comparison with published data.



Immediately  
after  
bacterial  
spray

15 minutes  
later

30 minutes  
later

60 minutes  
later

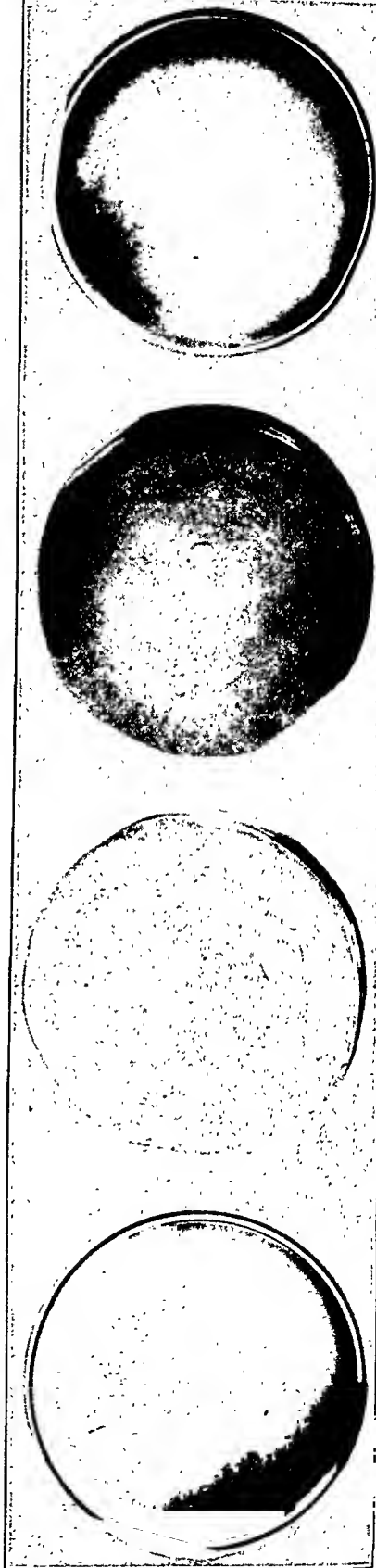


FIG. 2.—Bacterial plates showing colony counts (*Streptococcus C*) obtained in experiment shown in Table 3.

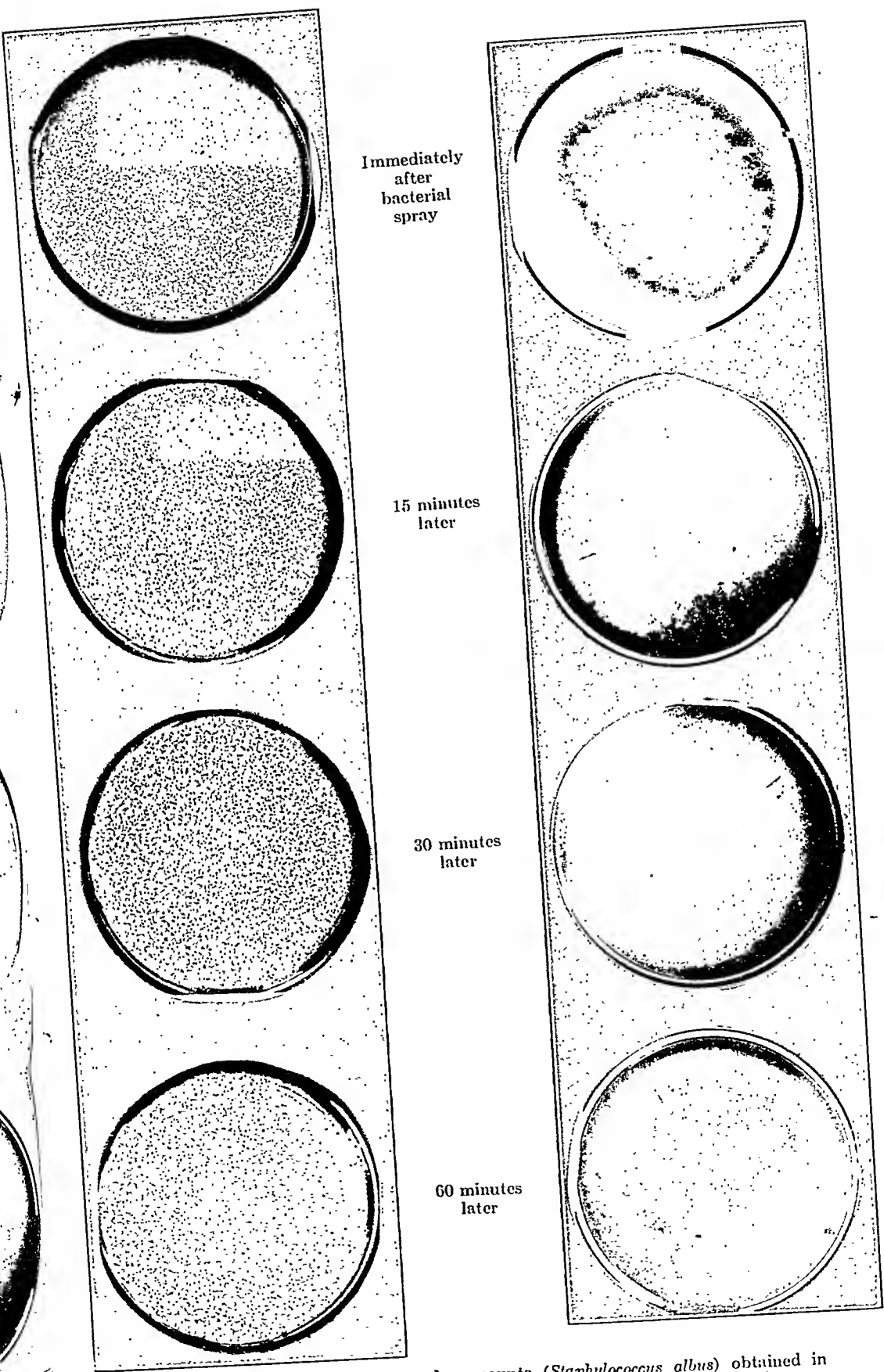


FIG. 3.—Bacterial plates showing colony counts (*Staphylococcus albus*) obtained in experiment shown in Table 4. (367)

this amount may be reduced in many installations. If this untreated air is introduced in the duct system it not only affords fresh air for the occupants but enables thorough mixing of the glycol vapor with it before it is distributed through the blowers into the room. Thus we would have a room relatively air-tight with all outside air coming into the space being controlled for adequate volume and glycol concentration.

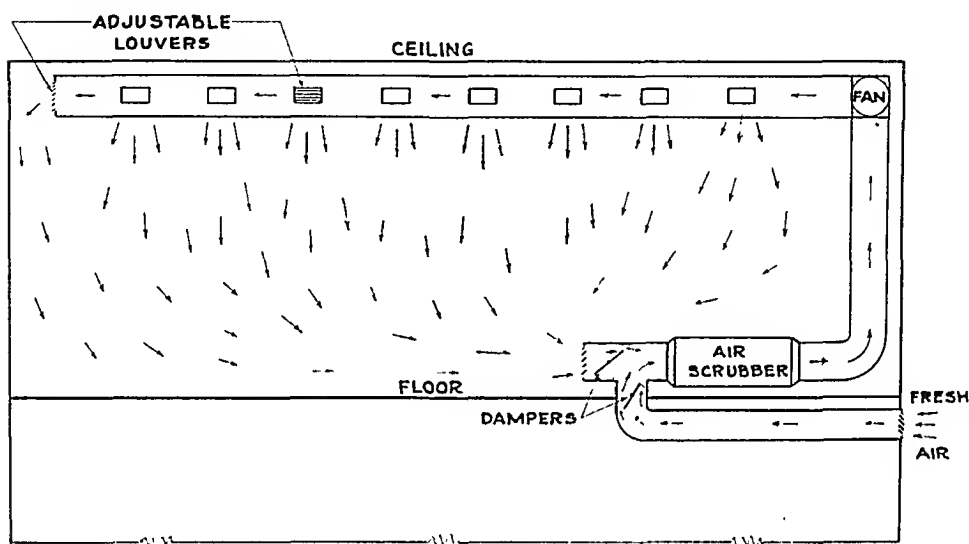


FIG. 4.—Layout of duct system for distribution of glycol vapor.

When glycol vapor is introduced into a space the desired concentration may be readily attained. The problem then to be met is the maintenance of this concentration in the room. It has been found that many factors operate to cause a decrease in this concentration. The most important is the normal air exchange, infiltration and exfiltration, that takes place in any space. Even in especially built chambers constructed so that all possible points of egress are sealed it is difficult to prevent air exchange. In normal living spaces under windy conditions this air exchange may be as high as 8 changes per hour, depending upon the tightness of construction and the number and locations of doors and windows. Normally the air changes per hour lie in the range from  $\frac{1}{2}$  to 3.

Other causes for a decrease in concentration are condensation of the vapor on cold surfaces, absorption of the vapor by the clothing of the occupants and other surfaces in the room, and the actual metabolism of glycol which might occur by the occupants breathing the air.

During the course of our experiments we found that when air supersaturated with glycol was introduced a fog occurred in the room.\* This phenomenon occurred more readily when the temperature was low and humidity was high in the space. When the concentration of

\* In the course of a study on the possible fire hazard produced by the glycols it has been found that this supersaturated air is not without danger. These data are in press.

propylene glycol was over 0.5 mg. per liter, or the concentration of triethylene glycol reached a level of about 0.01 mg. per liter, fog resulted. This, of course, is objectionable in occupied rooms and limits the method of glycol introduction as well as necessitating careful control of concentration. Further observations on fog formation are being carried out.

Thus the actual rate of introduction of glycol into any space must be determined for each space to be treated. We have developed several means for automatic control which will be reported in subsequent publications.

The necessity for maintaining adequate humidity also raises certain difficulties in the problem of maintaining bactericidal activity. Since the greatest incidence of respiratory disease occurs during the cold periods of the year and since cold weather is accompanied by low relative humidities in heated spaces, water vapor as well as glycol vapor must be introduced into the space. If an air-conditioning unit is in operation this may readily be attained; however, since such apparatus is not usually available, moisture must be added in some other way. It may be introduced in the form of steam or may be produced along with the distribution of the glycol vapor.

**Summary and Conclusions.** 1. The production and maintenance of glycol vapor in adequate concentrations in large spaces is possible and readily attainable.

2. Glycol vapor diffuses freely throughout large spaces and no stratification of the gas occurs.

3. Some means of air agitation is desirable to insure adequate distribution.

4. Bactericidal activity occurs in large spaces as well as small test chambers.

5. Concentration of 0.2 mg. per liter of propylene glycol and 0.005 mg. per liter of triethylene glycol are necessary for immediate killing of the air-borne microorganisms. Lesser concentrations may suffice to control air-borne disease.

6. Maintenance of humidities in the region of 40% is necessary.

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## INFLAMMABILITY CHARACTERISTICS OF PROPYLENE GLYCOL AND TRIETHYLENE GLYCOL IN LIQUID AND VAPOR FORM\*

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THE recent observations on the effectiveness of certain glycol vapors in bringing about striking air sterilization present a most promising method for the control of airborne disease. It has been shown that the use of those substances, particularly propylene glycol and triethylene glycol, offers definite advantages over measures previously described. Bactericidal and virucidal concentrations of propylene or triethylene glycol vapor are odorless, non-toxic, effective in extreme dilutions, easy to distribute and reasonable in cost.<sup>1</sup>

In view of the possible widespread use of these materials as a means of prevention and control of airborne infection the following study was carried out to ascertain the fire hazard which might be created by their presence.

Since glycols are highly hygroscopic and freely miscible with water, one must assume that only rarely will water-free, 100% glycol be present except in storage. The glycol condensed on surfaces, that is, walls, ducts, windows, and so forth, will contain water, the proportion of water in the condensate being dependent upon the relative humidity and temperature of the space treated and the temperature of the condensing surfaces themselves.

**Propylene Glycol.** The physical properties of propylene glycol are as follows: Molecular weight, 76.06; boiling point, 368° F. at 14.7 p.s.i.; specific gravity, 1.0381; vapor pressure, 0.18 mm. Hg at 68° F.; and pounds per gallon, 8.64. Flash point data (furnished by the Carbide and Carbon Chemicals Corporation) determined by means of the open cup method, are as follows: 100% glycol flashes at 220° F.; 95% at 225° F.; 90% at 245° F.; 85% or lower—no flash point is evident.

**Experiments Conducted.** *Open Dish Tests.* Varying glycol-water solutions were placed in small evaporating dishes and the flame of a Bunsen burner was allowed to play over the surface of the fluid mixture. After about 60 seconds of heating, the contents of the dish containing 100% propylene glycol could be ignited and it burned with a blue flame until all of the liquid was consumed. A 90% mixture could also be ignited, although the flame had to be held over it for 2 or 3 minutes. An 80% mixture burned with even greater difficulty, requiring 4 to 5 minutes to ignite. Using 75% glycol, the mixture was ignited but after a few minutes the flame spontaneously went out. Mixtures containing 70% glycol or less could not be ignited unless they were violently boiled for a few minutes. It would appear that the more concentrated solution, resulting from the loss of water vapor, alone was inflammable.

\* The work described in this paper was done under a contract, recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Northwestern University.

*Asbestos Wick Tests.* Using a like series of concentrations as in the previous tests, asbestos wicks were wetted with the appropriate solution and ignited by means of a Bunsen burner flame. Results very similar to those described with the open dish were obtained; that is, as the amount of water in the solution was increased it became more and more difficult to ignite the wick. The 75% solution did not propagate flame, and lesser solutions were non-combustible.

*Match Tests.* When lighted matches were dropped into varying solutions, a slight flame about the match persisted for a few minutes with 100% glycol, but no flame occurred with solutions of 90% glycol or less.

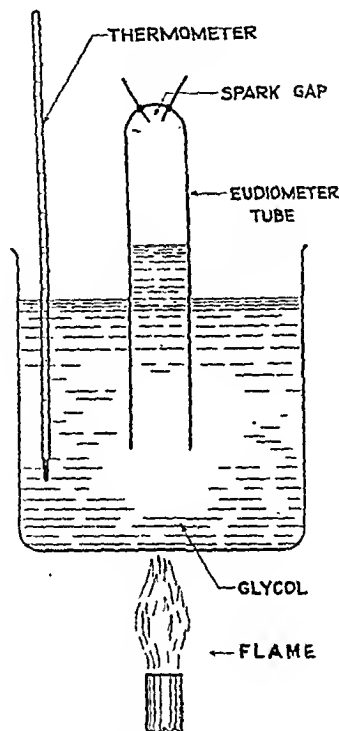


FIG. 1.—Eudiometer submerged in basin of glycol for vapor explosion test.

*Fabric Tests.* Silk, rayon, wool and cotton were immersed in varying solutions. If these materials were allowed to dry (to the touch) there was no increase in their combustibility. However, when wet with the solution they behaved as did the asbestos wicks except that in these cases the material itself also contributed to the combustion.

*Spark Tests.* It was impossible to ignite 100% glycol at room temperature by means of a spark produced by a spark coil.

*Eudiometer Studies.* A short eudiometer (Fig. 1) was arranged for immersion in a beaker containing 100% glycol solution. The eudiometer was partially filled with air and a spark was sent across the gap by means of a high-frequency induction coil. An explosion of the gas could not be obtained until the temperature of the liquid reached 214° F. If the liquid contained as little as 5% water, no explosion of the resulting vapor could be obtained. It was calculated that the concentration of propylene glycol in the explosive gas mixture was 85.5 mg. per liter.

*Flame Propagation of Unheated Glycol.* A film of 100% glycol was spread on a stone surface and a lighted match was placed at one end. The match burned for about 10 seconds, then went out spontaneously. A long stream of

the same material was also spread on a stone surface and again there was no spread of flame, the match going out after 60 seconds.

**Condensation Studies.** To determine the actual concentration of condensates occurring in conditions simulating field conditions, the apparatus shown in Figure 2 was constructed. Air was first humidified by bubbling it through water; it was then passed over an aqueous glycol solution which was warmed in a constant temperature bath. The resulting gases were then introduced into a glass chamber of 2 cu. ft. capacity. The humidity of the incoming air, as well as its glycol concentration, was regulated by changing the temperature of the water-bath or by increasing the percentage of water in the glycol-water solution. The atmosphere of the chamber was agitated by means of a small, 3-inch fan, and the humidity was determined by means of a wet-dry bulb thermometer. Two-liter samples of the chamber air were withdrawn for glycol analyses.

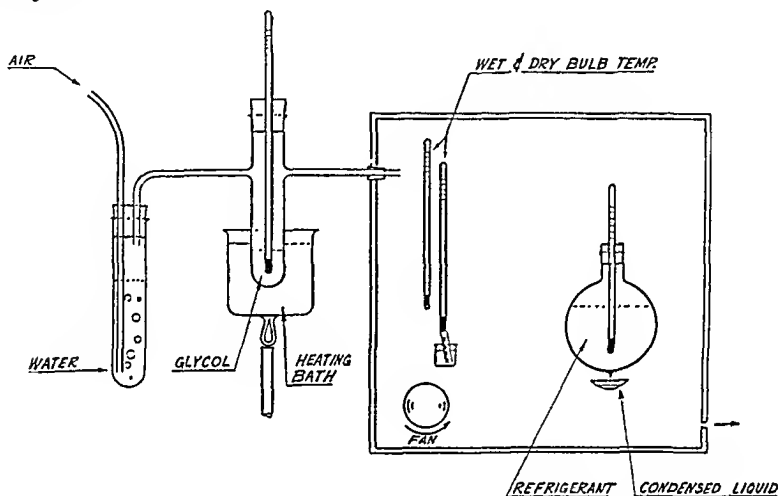


FIG. 2—Determination of concentration of condensates.

TABLE 1.—CONDENSATION STUDIES

Propylene glycol concentration (mg. per liter)	Chamber humidity (%)	Chamber temperature (°F.)	Condenser temperature (°F.)	Condensate concentration (%)
0.149 . . . . .	60	78	32	5.0 glycol
0.169 . . . . .	50	78	32	11.5
0.170 . . . . .	37	82	32	3.0
0.208 . . . . .	43	82	32	5.0
0.211 . . . . .	47	76	32	16.0
0.217 . . . . .	53	78	32	5.0
0.162 . . . . .	46	78	0-32	5.0
0.172 . . . . .	60	79	0-32	5.0
0.186 . . . . .	60	80	0-32	6.5
0.196 . . . . .	60	75	0-32	17.0
0.251 . . . . .	44	79	0-32	31.5
0.290 . . . . .	64	78	0-32	7.0
0.300 . . . . .	52	79	0-32	16.1
0.310 . . . . .	61	79	0-32	7.2
0.323 . . . . .	60	78	0-32	6.0

A standard 1-liter, round-bottomed flask was suspended in the chamber. A teat was fused to the bottom of the flask, allowing the condensate to drop into a watch crystal placed beneath. The flask was then filled with ice and, to obtain lower temperatures, with salt-ice mixtures. The samples of condensate were analyzed for glycol content by means of refractive indices. Observations are listed in Table 1. It may be seen that in a space, the humidity range

of which is 37 to 61%, with a temperature as high as 82° F., containing a condensing surface as low as -32° F., and filled with a maximum of 0.323 mg. of propylene glycol per liter, no inflammable condensate results. This is particularly striking since adequate bactericidal action occurs in air containing 0.1 mg. propylene glycol per liter.

**Triethylene Glycol.** The physical properties of triethylene glycol are as follows: Molecular weight, 150.17; boiling point, 548° F. at 14.7 p.s.i.; specific gravity, 1.1254; vapor pressure, 0.01 mm. Hg at 68° F.; and pounds per gallon, 9.37. Flash point data furnished by the Carbide and Carbon Chemicals Corporation, determined by means of the open cup method, are as follows: 100% glycol flashes at 330° F.; 95% at 345° F.; 90% or lower, no flash point is evident.

Tests on inflammability using the open dish method, flame propagation, fabric wicks, spark coil and matches, gave results practically identical with propylene glycol. In the eudiometer an explosion occurred at 356° F. with a calculated vapor concentration of 130 mg. per liter. If any water were present in the glycol no explosion could be obtained.

**Discussion.** Since the concentration of propylene glycol and triethylene glycol vapor necessary for bactericidal action is 0.1 mg. per liter and 0.005 mg. per liter, respectively, it is obvious from the above observations that there is no hazard produced by the presence of this quantity of vapor in the air. In the case of propylene glycol, 855 times this amount is necessary to produce an explosive mixture, and for triethylene glycol 26,000 times this quantity must be present. This explosive property, however, must be considered if an apparatus introducing superconcentrated gaseous mixtures is used, for at the point of exit of the gas such a concentration might possibly be attained.

The possibility of danger arising from the condensation of glycol on cold surfaces in the treated space must be considered even though, as has been shown, using the maximum glycol concentration plus a high humidity and low temperature on the condensing surface, very dilute aqueous solutions of glycol were obtained. It would appear that since relatively intense and prolonged application of heat is necessary to ignite solutions containing as little as 30% water, there is little chance for glycol condensates to burn in ordinary use. If, however, a fire should occur in such a treated space due to other causes, the heat produced would probably bring about the loss of water from the condensate and thus the glycol might add to the general conflagration, just as the other objects or material present, that is, woodwork, bed-clothing, and so forth.

Another source of danger would be at the area of storage of the glycol; here, too, if intense and prolonged heat were applied, fire might occur. It might be noted that this danger is no greater than that produced by the storage of other combustible materials, such as fuel oil. This hazard could be reduced by storing the glycol, not as 100% material, but as aqueous solutions containing as much as 20% water.

There would appear to be little difference between propylene glycol and triethylene glycol as far as fire hazard is concerned. According to the code of the New York City Fire Department, pure propylene glycol is classified as not combustible but inflammable, and triethylene as neither combustible nor inflammable. The smaller quantities of triethylene glycol necessary to produce sterilization of the air neces-

sarily results in a lesser amount of condensed glycol or surfaces. This is a matter of degree, particularly of time during which the substance is used.

**Conclusions.** 1. In the vapor-phase concentration required for air sterilization, propylene and triethylene glycol offer no fire or explosive hazard.

2. The addition of water to these substances greatly reduces the possible fire hazard produced by their presence in storage or vaporizing devices.

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### THROMBOPHLEBITIS AS A HITHERTO UNREPORTED COMPLICATION OF THIOCYANATE THERAPY OF HYPERTENSION

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IN the past 3 years we have treated 40 cases of essential hypertension with potassium thiocyanate.<sup>2</sup> These cases were carefully studied, including frequent blood thiocyanate levels, according to the method of Barker.<sup>1</sup> The clinical results of these studies are reported elsewhere.<sup>2</sup>

The purpose of this paper is to report on the occurrence of thrombophlebitis as an apparently unpublished complication in the administration of potassium thiocyanate.

Of the 40 patients treated, 4 exhibited this complication. Such high incidence makes us feel that it is not a mere coincidental finding but a definite toxic effect.

The usual toxic effects that have been reported are dermatitis, weakness, dizziness, loss of appetite, and easy fatigability. Angina pectoris in the course of therapy has also been reported.<sup>3</sup> We have observed 1 case of coronary thrombosis in a patient taking the drug, but this complication is not so very unusual in the course of untreated hypertension. Venous thromboses in the extremities have never, to our knowledge, been previously reported.

**Case Studies.** Of the 4 cases observed we shall report in detail the following one:

**CASE 1.** B. R., a white male, age 51, was first seen in the clinic on Dec. 28, 1937. He was admitted with complaints of generalized throbbing headaches, poor memory, dizziness, dyspnea, orthopnea, palpitation, precordial pain, nocturia and fatigue.

The blood pressure on admission was 238/130. A 6-foot film revealed the heart to be enlarged in all diameters, accentuation of the left ventricular curve, and dilatation and tortuosity of the aorta. The electrocardiogram showed deviation of the electrical axis to the left. A pyelogram made at another hospital had been reported as showing some shagginess of the calyces

of the right pelvis. Intravenous pyelography performed when patient was admitted to the ward revealed both kidneys to be normal in size, shape and position. The pelves, calyces and ureters were normal bilaterally. The blood Wassermann was negative, the hemoglobin 85%, red blood cells 4.65 million, white cells 6600 per c.mm., with 80% polys and 20% lymphocytes. The non-protein nitrogen of the blood was 31 mg. per 100 cc., the creatinine 2, and the uric acid 3.6. The urine revealed a specific gravity of 1.018 and contained a trace of albumin. Occasional hyaline and granular casts were seen on microscopic examination, as well as an occasional red blood cell and 2 to 3 white cells per high-power field.

After a period of observation of slightly over 3 years, during which time the blood pressure was persistently at a high level with the systolic at 190 to 260 mm. Hg and the diastolic at 130 to 150 mm. Hg, he was started on potassium thiocyanate therapy. The initial dose was 0.1 gm. 3 times a day, and after a week a blood level of 4 mg. thiocyanate per 100 cc. was reached. The blood pressure, however, remained at 257/140. On the same dose, a week later, the blood pressure was 232/140 and the blood thiocyanate, 4.2 mg. per 100 cc.

A slight dermatitis of the scalp was noted but it was decided to continue the thiocyanate therapy and the dose was increased to 0.2 gm. 3 times daily. A week later the determination of thiocyanate in the blood showed a level of 6.9 mg. per 100 cc., but the blood pressure remained elevated, the systolic being 226 and the diastolic 130. The dermatitis had cleared and the patient claimed to be subjectively improved, stating that the severe headaches had abated. The dose was increased again to 0.3 gm. thrice daily. There was no increase in the blood thiocyanate, the concentration remaining at 6.45 mg. per 100 cc. The blood pressure was still high so he was given 0.4 gm. 3 times a day, a total of 1.2 gm. daily. This time a blood level of 10.8 mg. was reached. There was no perceptible lowering of the blood pressure. The dose of 1.2 gm. daily was continued for 2 weeks when he began to show some nausea and loss of appetite and it was decreased to 0.3 gm. daily, lowering the concentration of thiocyanate in the blood to 5.5 mg. per 100 cc. The blood pressure had not been lowered at all but the patient stated that he felt better while on the medicine. It was therefore continued. After having received thiocyanate for 6 months he developed a thrombophlebitis in the left leg. Since there had been no objective evidence of clinical response to the thiocyanate therapy, the drug was discontinued and the phlebitis treated with rest and cold application. The acute inflammatory symptoms subsided readily, but the superficial veins could be palpated as hard cords.

Now the patient began to complain of a return of his headaches, dizziness, and visual disturbances consisting of seeing "snow falling." When all evidence of acute inflammation had subsided the thiocyanate treatment was re-instituted and within a month he presented evidence of phlebitis in the other leg. The thiocyanate was again discontinued, and started again after acute inflammation had subsided. Four months later there was a reappearance of the phlebitis in the right leg and also evidence of inflammation and thrombosis in and around the veins of the right arm and forearm. This time the drug was discontinued and has not been resumed. The patient is still under treatment and is running a consistently high blood pressure of about 250 mm. Hg systolic, and 150 mm. Hg diastolic.

In addition to the above case thrombophlebitis was also encountered in 3 other cases in our series. Short summaries of these cases are herewith presented:

CASE 2.—M. J., a colored female, age 64, was started on potassium thiocyanate on Oct. 11, 1940. The blood pressure at the start of treatment was 230/120. On a dose of 0.1 gm. 3 times a day the blood thiocyanate level was 19 mg. per 100 cc. and it was decreased to 0.1 gm. twice daily, when the concentration in the blood was reduced to 12.5 mg. On April 8, 1941, the blood

pressure had been reduced to 170/110, but a phlebitis of the veins about the left ankle was discovered and the drug was discontinued.

CASE 3. M. D., a white female, age 52, was taking thiocyanate for a period of 3 years with a satisfactory lowering of her blood pressure, and maintaining a blood thiocyanate level between 8 and 10 mg. On January 26, 1943, approximately 3 years after she had started taking thiocyanate, there developed a swelling of the left leg with patchy areas of redness and tenderness and the drug was discontinued.

CASE 4.—H. S., a white male, age 71, was given potassium thiocyanate in a dose of 0.1 gm. 3 times a day and after 1 week showed evidence of thrombophlebitis. The drug was discontinued before any blood level was obtained.

**Summary and Conclusions.** Four cases of thrombophlebitis are reported as occurring in the course of therapy of hypertension with thiocyanate, an incidence of 10%. This high frequency is much greater than can be explained on pure coincidence and must be accepted as a toxic effect which has not been previously reported.

It does not appear to be related to the level of thiocyanate in the blood, and may occur early or late in the course of treatment.

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### A CASE OF ACUTE MENINGITIS CAUSED BY NEISSERIA PERFLAVA

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In the winter and spring of this year, meningococcus meningitis assumed epidemic proportions and a considerable number of cases was seen at this hospital. The effectiveness of sulfonamide was striking. Consequently, it became important to us to investigate thoroughly any cases in which therapy failed. One such case was seen early in its course, was treated with large doses of sulfapyridine and finally with antimeningococcus serum and, nevertheless, resulted in a fatality. Cultural examination of the organism obtained from the spinal fluid has demonstrated that it is not a meningococcus but a chromogenic diplococcus of the pharyngeal group, and by its fermentation reactions is classified as *Neisseria perflava*. Cases of meningitis caused by pharyngeal *Neisseria* have been reported occasionally<sup>2,3,4</sup> and a group of 14 cases from one outbreak of meningitis was reported by Branham.<sup>1</sup> Since these organisms are serologically distinct from the meningococcus, antimeningococcus serum is ineffective. In our case, chemotherapy by the sulfonamide group was unsuccessful.

**Case Study.** R. J., a 31 year old merchant seaman, was admitted to the hospital late on the night of April 25, 1943. He seemed acutely ill, had hallucinations and tremor and gave a history of recent alcoholic intoxication. The nose and throat were congested and the temperature was 37.6° C. During the following 24 hours, his mental condition became worse and he began

to thrash around restlessly with rolling eyes. Examination did not reveal any injury to the head or body. The pulse was 140 and temperature 38.2. Several small red spots (petechiae) were noted in the skin of the abdomen and chest. Positive Babinski, Oppenheim and Gordon signs were found on the left side, a partial Babinski on the right side and the Brudzinski tests were positive on both sides. There was extreme rigidity of the neck and the Kernig sign was positive. The patient responded only to painful stimuli.

Spinal tap was done on April 27. The fluid was cloudy and greenish. The initial pressure was 220 cm. This fluid revealed cell count of 15,000, mostly neutrophils. Gram-negative intracellular diplococci were present. These organisms appeared larger than the usual strain of *Neisseria intracellularis*. Six gm. of sulfapyridine were administered intravenously followed by 1 gm. every 4 hours for a total of 21 gm. During the next 24 hours, he also received 120 cc. of antimeningococcus serum. A second spinal tap revealed increased cloudiness of the fluid. The color was greenish-yellow and the pressure 250 cm. Since the clinical condition was deteriorating, 20 cc. of antimeningococcus serum was administered intraspinally on April 29. The temperature continued to rise on the 29th, passing 40° C., and on the 30th, reached 41.8°. Death occurred late that night, 5 days after admission.

Autopsy revealed no essential morbid processes except in the head. A very thick, greenish-yellow exudate was found closely attached to the base of the brain from the region of the optic chiasm back to the medulla. In places, the exudate was 1.5 cm. thick and on section revealed cavitation with free pus enclosed in the pia-arachnoid.

In the routine examination of the base of the skull, it was apparent that the thickest exudate was localized around the pituitary. When this was removed, an oval fenestration in the floor of the pituitary fossa was revealed, measuring approximately 4 mm. in diameter. Its edges were smooth and through it the interior of the sphenoid sinus was plainly visible. No other lesions or defects of the skull were found. The contents of the sphenoid sinus consisted of thin mucus without gross pus.

Microscopic examination showed an unusually dense exudate of neutrophils in the pia and arachnoid. There was no extension of the exudate around the cortical vessels. A striking finding was the presence of masses of exudate with necrosis and the formation of small abscesses in the anterior lobe of the pituitary. This unusual site and the intensity of the inflammatory process in this area supported the conjecture that the portal of entry was the defect in the floor of the pituitary fossa.

The spinal fluid and the exudate at the base of the brain were cultured on the modified McLeod medium used for *Neisseria* in this laboratory. An abundant growth of greenish-yellow, slightly raised discrete colonies developed in 24 hours. Even at this early period, the chromogenesis was apparent. Fermentation was noted in media containing dextrose, maltose, levulose, sucrose and mannitol. The organism showed no agglutination by polyvalent anti-meningococcus serum.

**Discussion.** Branham,<sup>1</sup> commenting on the significance of atypical *Neisseria* as the causative agents of meningitis, states, "The natural habitat of the genus *Neisseria*, other than the gonococcus, is the nasopharynx. Meningeal invasion by any of these other than the meningococcus is only of occasional occurrence." However, her report of 14 cases caused by a homogeneous group of chromogenic *Neisseria* leads one to believe that the pathogenic activity of these organisms outweighs their biochemical and cultural peculiarities in establishing their identity as meningococci. On the other hand, Wilson and Smith<sup>5</sup> suggest that all *Neisseria* differing from typical meningococci and gonococci be placed together in one species, *Neisseria pharyngis*. The problem of classifying atypical Gram-negative diplococci is quite



different in meningitis from what it is in case-finding studies in gonorrhea. Although gonococci exhibit constant morphologic and biochemical characteristics, meningococci show variations in regard to fermentation and antigenic composition. Reimann and Koucky<sup>4</sup> cultured from a case of meningitis an organism which was chromogenic, produced acid in glucose and lactose and was not agglutinated by polyvalent antimeningococcus serum. They regard these deviations as "part of the pattern of variation of the bacterium concerned." It seems to us that the inclusion among *Neisseria intracellularis* of organisms differing in fundamental characteristics from the recognized strains leads to confusion. The application of bacteriologic or other scientific systems to medicine is pragmatic, and for the physician the difference between an organism susceptible to a specific serum or chemical and another, however closely related one, insusceptible to these agents is essential.

**Summary.** A case of acute meningitis is described differing clinically from meningococcus meningitis in failure to respond to sulfonamide and serum therapy. The causative organism was *Neisseria perflava*. It appears that the unusual nature of the invading organism might have been suspected from the pigmentation of the spinal fluid. The portal of entry found at autopsy was a congenital defect in the roof of the sphenoid sinus.

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### A CASE OF HISTOPLASMOSIS (DARLING) WITH AUTOPSY

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AND

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HISTOPLASMOSIS was first described by Darling in 1906 and 1908. Since that time the diagnosis has been made with increasing frequency. In this report the literature will not be reviewed, as this has been done recently by Meleney,<sup>2</sup> Wright and Hachtel,<sup>7</sup> and others, and the cultural characteristics adequately covered by De Monbreun.<sup>1</sup> To those cases already reported in the literature the authors wish to add another.

**Case Report.** (A-68-42, A.M.M. Acc. 89561, Fort Benning). C. L. P., a 23 year old colored male, a laborer from Alabama, was admitted to the Station Hospital, Fort Benning, Ga., on Oct. 22, 1942. Before coming under observation he had been acutely ill 7 weeks with chills, fever, aching, cough, and blood-streaked sputum. The patient stated that he felt fairly well during the day, but each evening would have chills, fever, aching, and sweats. The cough

with blood-streaked sputum had been present for about a year. The sputum was described as thick, milky white, and occasionally streaked with blood. There had been a 15 pound weight loss in the 7 weeks prior to admission. Shortness of breath had been noticeable for about 2 weeks.

The *family history* was non-contributory. The *past history* was significant in that the patient had had malaria in 1938, and typhoid fever in 1935.

*Examination* revealed a colored adult male who appeared to be acutely and seriously ill. He appeared toxic and dyspneic. The conjunctivæ were clear; the mucous membranes appeared pale. The tonsils were small; the mouth was foul. No lymph nodes were palpable. The chest was symmetrical, expansion free, but excursions were small. Fremitus over both apices was diminished and the percussion note impaired. Breath sounds were distant, and numerous moist râles were scattered over both lungs. Respirations were 30 per minute. The heart showed no abnormalities except for a tachycardia of 120 per minute. The blood pressure was 94/66 mm. of mercury. The abdomen was distended and tympanitic. The upper abdomen was tender. The liver and spleen extended 2 and 4 fingers breadth below the costal margin respectively. The spleen was tender. The superficial and deep reflexes were bilaterally equal and active.

*Laboratory Data.* Blood count (10-25-42): R.B.C., 3,100,000 (hemoglobin, 60%); W.B.C., 5000 (polymorphonuclears, 75%; lymphocytes, 23%; monocytes, 2%). No malarial parasites seen on 3 examinations. *Kahn* test negative. *Sputum* examinations on 9 occasions failed to show acid-fast organisms. *Sedimentation* rate (10-25-42) was 27 mm. in 1 hour (Cutler). *Urinalysis* was essentially negative except for a trace of albumin; specific gravity, 1.010. *Feces* examination was negative for parasites and ova; occult blood was present.

*Roentgen ray examination* (10-22-42) showed an extensive infiltration of both lungs, miliary in type. Diagnosis: pulmonary tuberculosis, miliary. Re-examination showed the same picture on 10-24-42.

*Clinical Course.* During the 1st week of hospitalization, the patient had recurring chills every evening with rise of temperature to 104° F., followed in 7 hours by a drenching sweat. The 2nd week the evening temperature only spiked to 102° F., with the chills and sweats as before. The distention of the abdomen was very difficult to control for the 1st week, after which the patient was somewhat more comfortable. The cough and blood-streaked sputum persisted; the dyspnea became progressively worse. The lung findings changed gradually each day until 6 days before death, at which time the percussion note was flat throughout. Breath sounds were barely audible, and no râles were present. The pulse rate became very irregular as a result of numerous premature contractions and was almost imperceptible during the last 6 days. Terminally, the respiratory rate rose to 40 per minute. Restlessness and dyspnea were marked. Coma and death occurred on the 13th hospital day.

*Postmortem Examination* (15 hours after death). The body was that of a negro male, 23 years of age, fairly well developed, but emaciated, measuring 64.5 inches in length, and weighing approximately 90 pounds. The head showed nothing of significance other than poor oral hygiene and slight oozing of bloody fluid from the nose. A few small lymph nodes were found in the left axilla. The omentum was adherent to an enlarged spleen. The peritoneum was smooth otherwise, and there was 100 cc. of clear yellow ascitic fluid. Both lungs filled their respective pleural cavities. There were tough fibrous adhesions posteriorly on both sides, as well as on the diaphragmatic surface and apex of the right lung. All lung fissures were virtually obliterated with adhesions. There was a small amount of cloudy fluid in each pleural cavity and a rather diffuse recent fibrinous reaction. The pericardial sac was thin and contained a small amount of clear yellow fluid. The heart was approximately normal in size.

The left and right lungs weighed 1080 and 1130 gm. respectively. Both pleural surfaces were roughened by numerous fibrous and fibrinous adhesions. All lobes were very firm and presented many areas of increased density.

Crepitus was markedly reduced. The cut surfaces of all lobes were similar. They were firm, dirty yellowish gray, with a few small scattered areas of red, relatively normal lung. One 2 x 3 cm. area in the subapical portion of the right upper lobe showed markedly increased density. Fibrosis was generally increased throughout, but no calcium was encountered. The hilar lymph nodes were not prominent. There was no cavitation.

The heart weighed 300 gm. and presented nothing of note grossly.

The spleen weighed 650 gm. The external surface was grayish red and presented shaggy fibrous tags over its upper portion. The pulp was exceptionally firm and light red. It was scraped with difficulty; the normal architecture was obliterated.

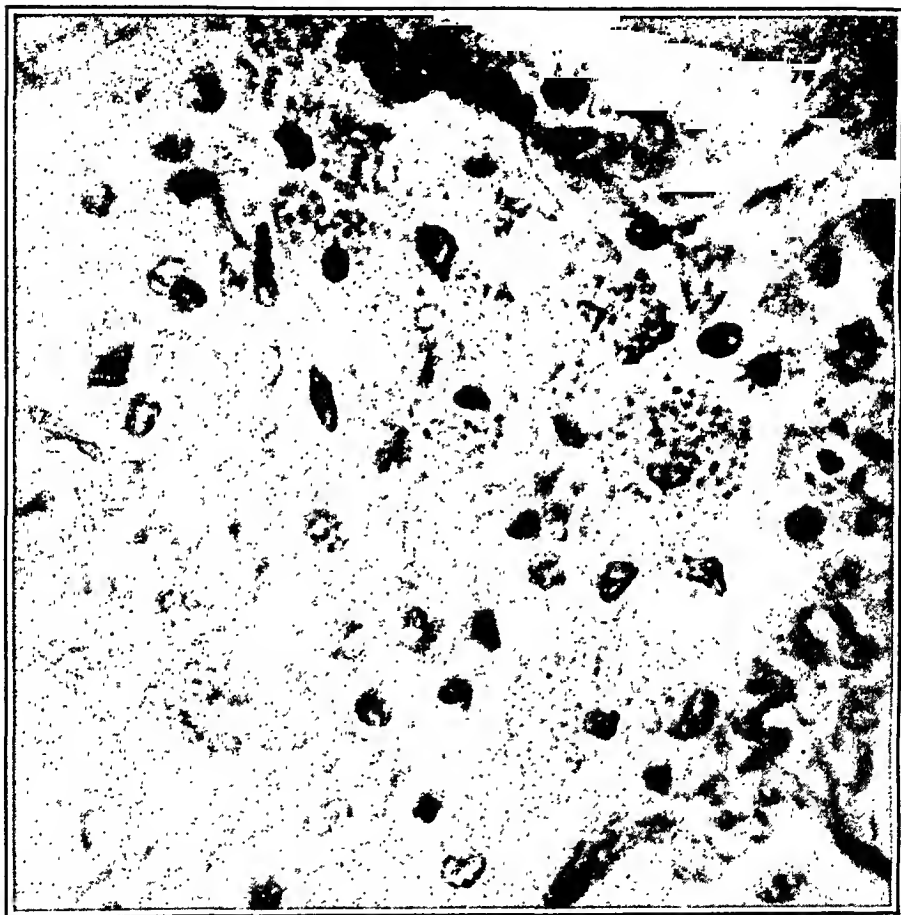


FIG. 1. Lung (440 X): Pulmonary alveoli filled with parasite-laden macrophages. Fibroblastic activity is also evident.

The liver weighed 2040 gm. Its external surface was smooth and presented numerous small, firm, circumscribed, grayish white areas varying in size from 1 to 2 mm. These areas were not calcified. The cut surface showed little of note other than an occasional small rounded area such as presented on the external surface.

The gall bladder, biliary duct system, adrenals, and gastro-intestinal tract showed nothing of note. The pancreas weighed 60 gm. and revealed no gross findings.

The peripancreatic and portal lymph nodes were enlarged, varying from 1 to

2.5 cm. in diameter. They were firm and presented a homogeneous yellowish gray cut surface.

The left and right kidneys weighed 210 and 195 gm. respectively. Their capsules were thin, but stripped with some difficulty, revealing a granular reddish brown cortical surface. Consistency was moderately increased, but the cut surface presented normal markings.

*Microscopic Examination. Lungs:* The primary lesion appears to be a nodular area of consolidation resulting from numerous large parasite-laden macrophages filling the alveolar spaces. The parasites are round to ovoid encapsulated structures about one-third to half the size of a red cell and present a central Gram-positive polar staining ovoid "nucleus." The nodular

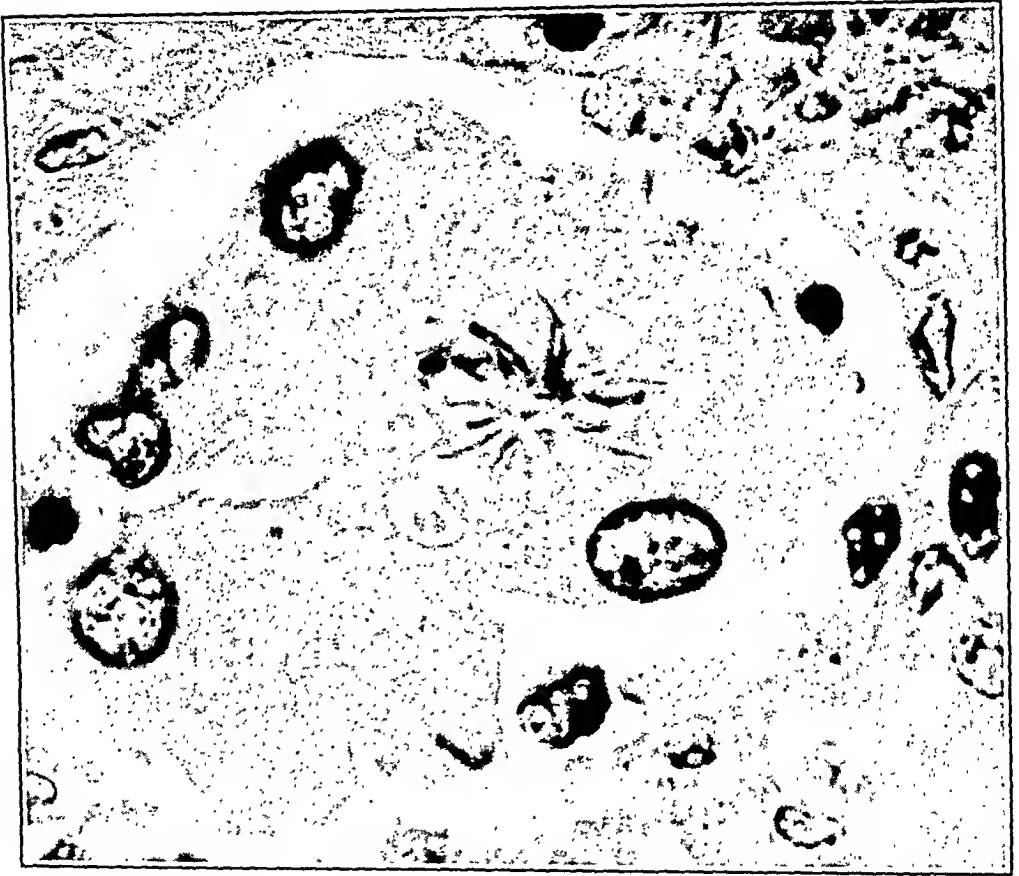


FIG. 2.—*Spleen* (950  $\times$ —oil immersion): "Asteroid" inclusion forms in Langhans' giant cells.

areas exhibit a marked tendency towards confluence. The larger lesions thus formed have large zones of central necrosis which still retain the "ghost-like" architecture of the lung structure. The pulmonary vessels at these sites exhibit a rather marked obliterative endarteritic process which would appear to have contributed to the necrosis. Surrounding the necrotic areas there is a rather marked proliferation of fibroblasts obliterating the air spaces and enclosing myriads of parasite-laden macrophages. In such granulomatous areas numbers of necrotic neutrophils can frequently be found. Surrounding the nodules the lung parenchyma exhibits complemental emphysema and mild fibrous septal thickening. In some sections, presumably from the subapical area, there are small wedge-shaped areas of dense subpleural scarring resemb-

ling the common-subapical scars described in association with a healed reinfection type of tuberculosis. There is a moderate degree of parenchymal fibrosis too, which appears to be entirely independent of the principal process already described. However, the scarred areas accentuate the dilated lymphatic vessels which contain numerous and often parasite-laden macrophages (Fig. 1).

*Liver:* Principally in the subcapsular area, but also involving many of the portal spaces, there are variably-sized granulomatous nodules which compress and distort the adjacent liver parenchyma. The nodules contain frequent parasite-laden macrophages and are mildly infiltrated with round cells. The remaining bulk of the liver tissue preserves its normal lobulation which is accentuated by moderate sinusoidal dilation and congestion. The Kupffer cells are hypertrophied and often contain numbers of organisms.

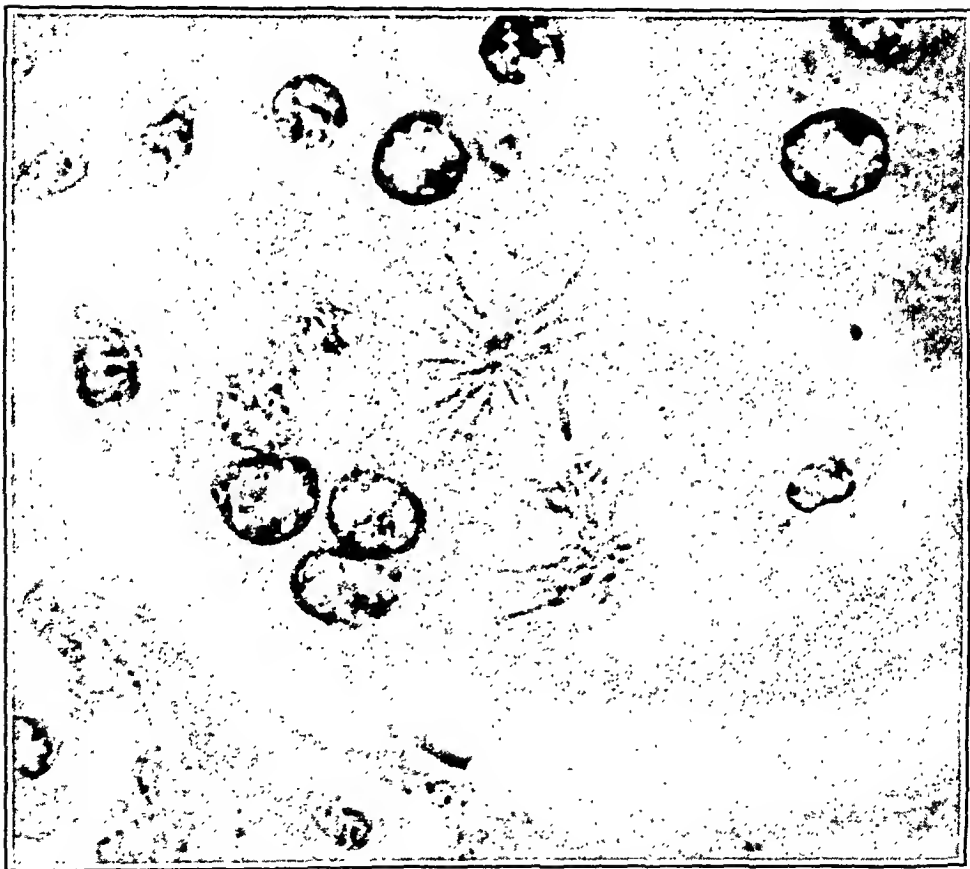


FIG. 3.—Spleen (950  $\times$ —oil immersion): "Asteroid" inclusion forms in Langhans' giant cells.

*Spleen:* There is a marked alteration of the splenic architecture by marked fibrosis along the vascular tree and trabecular framework. The broad bands of fibrous tissue thus formed are featured by the presence of numbers of large Langhans' type giant cells. Lymphoid follicles are largely obliterated. The pulp persists as islets of sinusoids between large fibrous bands. Although organisms may be demonstrated in macrophages present in the sinusoids, they are comparatively few in number. The Langhans' giant cells often contain one or more prominent amphophilic and Gram-positive stellate or "asteroid bodies," which are contained in a scalloped, irregular vacuolar space. The appearance resembles a flower whose petals are formed by clusters of what

appear to be clear ovoid bodies and whose central portion is amphophilic. Radially arranged, slightly tortuous bands of similar staining reaction appear to be dispersed amongst the ovoid bodies. However, selected asteroid bodies clearly show the presence of an unstained capsule covering each arm. It may be that their overlapping produces the effect of clustered ovoid bodies, but in many instances such clusters can be demonstrated in the absence of an asteroid. These ovoid bodies have amphophilic "nuclei" and resemble the organisms seen in other tissues. However, they frequently fail to retain the Gram stain (Figs. 2 and 3).

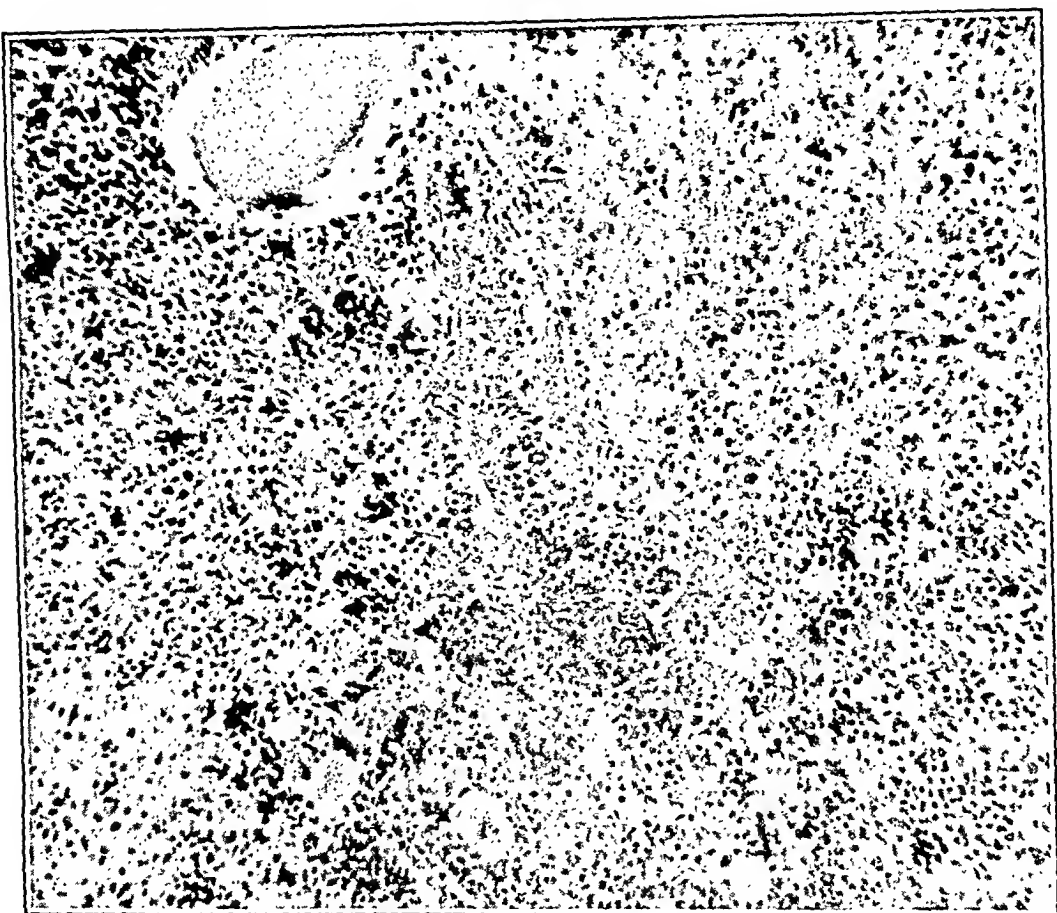


FIG. 4.—*Bone marrow* (440 X): Granulomatous foci displace and encroach upon the functional hematopoietic marrow which presents as a wedge in the center of the field. Anemia and leukopenia are thereby explicable on a myelophthisic basis.

*Lymph Nodes:* There is a marked alteration of the nodal architecture by an enormous hypertrophy of the reticulo-endothelial cells and by fibrosis and hyalinization of the lymphoid cords. Numerous cells of the macrophage variety fill and distend the sinusoids often forming nodular clusters. Numbers of ovoid intracellular organisms, as previously described, may be demonstrated.

*Kidneys:* Intertubular round and plasma cell infiltrates are prominent. The renal tubules are moderately separated by the interstitial infiltration and edema and exhibit rather marked parenchymatous degeneration.

*Pancreas and skeletal muscle:* Not noteworthy.

*Heart:* There is a mild rather patchy increase of mononuclear cells in the interstitial stroma. The muscle fibers are of average size with clearly defined striations.

*Bone marrow:* Sections of the rib marrow show numerous granulomatous foci which largely displace a qualitatively hyperactive hematopoietic tissue. Numbers of organism-laden macrophages may be demonstrated in the granulomas. It is quite evident that there is a very marked quantitative decrease of the functioning blood-forming tissues (Fig. 4).

*Miscellaneous:* Occasional giant cells of the Langhans' variety may be demonstrated in granulomatous areas of the lung, liver, lymph node, and bone marrow, as well as in the spleen as described above. "Asteroid forms" can be demonstrated in the giant cells at any of these sites.

**Comment.** The publication of over 30 cases since 1938 when only 7 cases were reported prior to that time would indicate that histoplasmosis, although not common, is perhaps not as rare as was once thought. Histoplasmosis may be suspected when a patient presents continued fever, leukopenia, splenomegaly, hepatomegaly, anemia, lymphadenopathy, skin lesions, and gastro-intestinal ulcerations. Clinically the last three features were not present in the case reported herein. Obviously the more common diseases must be ruled out before considering the rareties. In the present case the clinical picture was confused by a Roentgen ray diagnosis of miliary tuberculosis, with which the finding of 9 sputa negative for acid-fast organisms was not incompatible. Hindsight, in the present case, would indicate that the fungus was probably present in the sputum, and if suspected, could have been demonstrated by smear or culture. Again, more careful blood studies, cultures, or bone marrow examinations could have led to an antemortem diagnosis as made in some of the previously reported cases. Biopsy of enlarged lymph nodes or of granulomatous skin, nasopharyngeal, or gastro-intestinal lesions constitutes the remainder of the diagnostic armamentarium.

The presence of anemia and leukopenia in histoplasmosis may be beautifully explained by the bone marrow findings in the present case as a quantitative reduction of the hematopoietic marrow by multiple granulomatous lesions which displace it. This appears to be primarily a myelophthasic anemia, although secondary toxic effects cannot be ruled out. The presence of rather marked marrow hyperplasia between the granulomatous foci would speak against any marked toxic effect of the systemic infection.

Culturally the fungus grows as a yeast at 37° C. and produces mycelia with aerial hyphae at room temperature. Experimentally, both forms are pathogenic, but the mycelial form reverts back to the yeast form in tissues (Parsons).

"Asteroid forms" described in Langhans' giant cells in this case were previously described by Wolbach (1911). This author presented 5 cases wherein these cell inclusions were found. They were associated with disseminated granulomatous lesions of various sorts and involved lymph nodes in 3 cases; the lungs in 3 cases; and the spleen and liver in 2 cases. The primary pathologic diagnoses were diverse, and in all cases specific granulomata were carefully ruled out. For this reason the inclusions were not regarded as specific for any disease.\* The

\* Dr. Melvin Friedman, in our department, has recently observed a case not associated with histoplasmosis, where asteroid forms were so numerous that a detailed schema of their development could be constructed.—EDITOR.

staining reactions led Wolbach to hypothesize that these forms "are produced by etching of the fibrin reticulum which for some reason or other has acted as a foreign body, and has been taken up by endothelial leucocytes."

**Summary.** 1. A case of histoplasmosis occurring in a negro soldier from Alabama is reported.

2. The diagnosis was made by postmortem examination after a clinical diagnosis of miliary tuberculosis was made.

3. At the postmortem table a diffuse form of tuberculous pneumonia was suspected, and the correct diagnosis was not made until the microscopic sections were read.

4. Encapsulated "asteroid forms" are described in the granulomatous foci.

5. An explanation for the occurrence of anemia and leukopenia in this case is given.

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## HEPATORENAL FAILURE IN THE WATERHOUSE-FRIDERICHSEN SYNDROME\*

### CLINICO-PATHOLOGIC OBSERVATIONS IN TWO CASES WITH PROLONGED SURVIVAL PERIODS

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THE literature on the Waterhouse-Friderichsen syndrome has repeatedly stressed the concept of a distinct clinical entity characterized by an acute fulminating meningococcemia with adrenal hemorrhages and a rapidly fatal termination. A striking feature of this syndrome is the brevity of the clinical course, the majority of cases succumbing within 24 hours or less.<sup>1,4,5,7,8,10-12,16-18,20,21</sup> Levinson,<sup>13</sup> Lindsay, Rice, Selinger, and Robins,<sup>14</sup> Drummond and Tooke<sup>6</sup> have reported cases which have lived for periods as long as 48 hours but there are no reports in the literature of cases which survived beyond this time. Investigators unanimously report an invariably fatal outcome in the Waterhouse-Friderichsen syndrome with the single exception of Carey<sup>3</sup> who claimed a recovery.

This syndrome affects children primarily, 70% of the reported cases

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occurring under 2 years of age. Its rarity in adults has been emphasized by Aegerter,<sup>1</sup> who, in a review of the literature, found only 6 adult cases.

The paucity of clinical studies in this syndrome is due to the fulminating and fatal course so characteristic of these cases, which precludes any extended bedside observations. However, in the treatment of 134 cases of meningococcal infections by the authors<sup>16</sup> at the Station Hospital, Fort McClellan, Alabama, 5 cases of the Waterhouse-Friderichsen syndrome were encountered, of which 2 were unique because of their prolonged clinical courses. This paper is a presentation of hitherto unreported clinico-pathologic features observed by us in these 2 protracted cases. Our experience indicates that the cases of the Waterhouse-Friderichsen syndrome which survive the initial shock will succumb in a state of renal insufficiency directly attributable to severe hepatic necrosis. This combination of clinical and pathologic factors has been described as the hepatorenal syndrome or hepatorenal failure. Briefly, the hepatorenal syndrome is a process which terminates in death with uremic symptoms in its final phase clinically, and in which at necropsy, hepatic as well as renal damage is demonstrable.

The hepatorenal syndrome was first described by Heyd in 1923 to explain certain deaths following operative procedures on the external biliary system.<sup>9</sup> Since that time a voluminous literature has accumulated which has been excellently summarized by Boyce.<sup>2</sup> In a recent review of the literature, Reich<sup>10</sup> called attention to the occurrence of the hepatorenal syndrome in many diverse medical and surgical conditions, such as diphtheria, Addison's disease, toxemias of pregnancy, intestinal obstruction, biliary tract diseases and burns. Wilensky and Colp<sup>22</sup> have advanced the most satisfactory theory thus far to explain the hepatorenal syndrome. They postulate the existence of an initial lesion which affects the hepatic cells primarily and the renal nephron secondarily.

**Case Reports.** CASE 1. A. J. W., a 20 year old male, was admitted to the hospital on Jan. 24, 1943, complaining of a sore throat, cough, chills and fever. He denied any muscle pains, vomiting or headache. On admission, the physical examination was entirely negative except for a moderately inflamed pharynx. No nuchal rigidity was present and petechiæ were not observed. Temperature on admission was 102.8° F., pulse 90 and respirations 20. The diagnosis of an upper respiratory infection was made and symptomatic therapy instituted. His temperature fluctuated between 99° and 103° F. for the first 3 days and then gradually fell to normal on the 5th day. He remained afebrile until the evening of the 7th day when his temperature rose to 101.6° F. and he appeared acutely ill. His symptoms now were malaise, chilly sensations, headache and weakness, but no abnormal findings were noted on examination. A blood count showed 11,150 white blood cells. On the 8th day, the patient presented a dramatic change. He was desperately ill, pallid, apprehensive, restless and in severe shock. The sensorium was clear and the patient was well oriented. There were numerous petechiæ and purpuric lesions distributed over the entire body. Facial edema was observed particularly about the eyes. The pharynx was red and injected. Nuchal rigidity was absent and no abnormal neurologic reflexes were elicited. The temperature was 96° F., pulse imperceptible, respirations 28, and the blood pressure was unobtainable. A second blood count revealed 47,250 white blood cells with 94% polymorpho-

nuclears. Lumbar puncture was not done at this time due to the patient's critical condition.

A diagnosis of an overwhelming meningococcemia with adrenal hemorrhages (Waterhouse-Friderichsen syndrome) was made. The patient was placed in the antishock position and an infusion of 1500 cc. of 5% glucose in normal saline was given intravenously to which 30,000 units of meningococcus antitoxin were added. Following this infusion, the blood pressure rose to 60/40 and the pulse was 130 and of fair quality. One hour later, a 5% sodium sulfadiazine solution was given intravenously (5.5 gm. in 111 cc. of sterile distilled water). The blood sulfadiazine concentration 20 minutes later was 15.5 mg. per 100 cc. Massive oral doses of sulfadiazine were administered according to the following schedule: initial dose, 8 gm.; second dose 2 hours later, 5 gm.; third dose 3 hours later, 4 gm.; fourth dose 4 hours later, 4 gm.; and 3 gm. every 4 hours for the next 3 doses. Thereafter dosage was regulated to maintain the blood concentration between 15 and 20 mg. On this régime the patient appeared to rally. A total of 80,000 units of meningococcus antitoxin was given intravenously within the first 18 hours of therapy. To combat the shock and impaired peripheral circulation, 500 cc. of blood plasma was given. The blood pressure rose to 80/60. Adequate amounts of fluid and periodic injections of desoxycorticosterone intramuscularly were administered.

At the onset of treatment and throughout the patient's course, there was a marked disturbance of renal function which was characterized by complete anuria for the first 24 hours followed by oliguria persisting until death. The specimens of urine obtained revealed no abnormal changes. In addition, the patient was incontinent of feces and passed many brown liquid stools until he succumbed.

On the 9th hospital day, the patient was still conscious and oriented but the respirations were rapid, labored, and shallow. Cyanosis was absent. Many bubbling râles were heard over the entire chest. The edema of the face persisted, but was not found in any other part of the body. The blood pressure remained at 80/60. The temperature was 98.4° F., pulse 110 and respirations 36. The blood sulfadiazine level was 14.4 mg. per 100 cc. On lumbar puncture, a crystal clear spinal fluid under normal pressure was obtained which was negative on examination. Colonies of *Neisseria intracellularis* Type II-IIa were grown from the blood culture. An additional 500 cc. of plasma was given. Because of dyspnea, the patient was placed in an oxygen tent.

On the 10th hospital day, the patient became stuporous but responded to painful stimuli. Bubbling râles were still audible. Temperature was 98° F., pulse 90 and respirations 40. Blood pressure was maintained at 80/60. Blood sulfadiazine concentration was reported as 19.5 mg. The non-protein nitrogen was 86. Despite all therapy, the course of the disease continued unfavorably, and the patient succumbed on the 11th hospital day, 80 hours after the onset of the Waterhouse-Friderichsen syndrome.

*Autopsy.* The body was that of a well-developed, thin young man. A moderate number of petechiæ were scattered over the body. Four hundred cc. of a clear, straw-colored fluid was present in each pleural cavity and 1000 cc. of a similar fluid in the abdominal cavity. This fluid clotted spontaneously during the course of the autopsy. The heart was enlarged and the right auricle was dilated. The liver weighed 2035 gm. and, on section, revealed moderate congestion. The kidneys appeared normal, the right weighing 185 gm. and the left 170 gm. The capsule stripped with ease, revealing a smooth, red-brown surface. The cortico-medullary demarcations were distinct. No exudate was present in the brain and the ventricles contained clear cerebrospinal fluid. The most striking finding was noted in the adrenal glands, which weighed 15 gm. together. Both adrenals were firm and in normal position but showed hemorrhagic areas involving the left more than the right.

Microscopic examination showed histologic changes in the adrenals, liver and kidney. The adrenals showed hemorrhages of varying sizes involving both the cortical and medullary areas. The general architecture of the liver was normal. There was central vein and sinusoidal engorgement. In the

central zone, there was marked dissociation of liver cords, many of the cells showed necrobiosis and many were heavily laden with bile pigment. The mid-zone showed necrobiosis and vacuolization. The liver cells in the peripheral zone showed no noteworthy change. In the portal areas there was a moderately intense diffuse polymorphonuclear and lymphocytic infiltration with mild edema. The kidney showed increased cellularity and avascularity of the glomerular tufts. Focal areas of polymorphonuclear infiltration and edema interstitially were observed. There was cloudy swelling of the convoluted tubular epithelium and albuminous deposits were present in the tubular lumina.

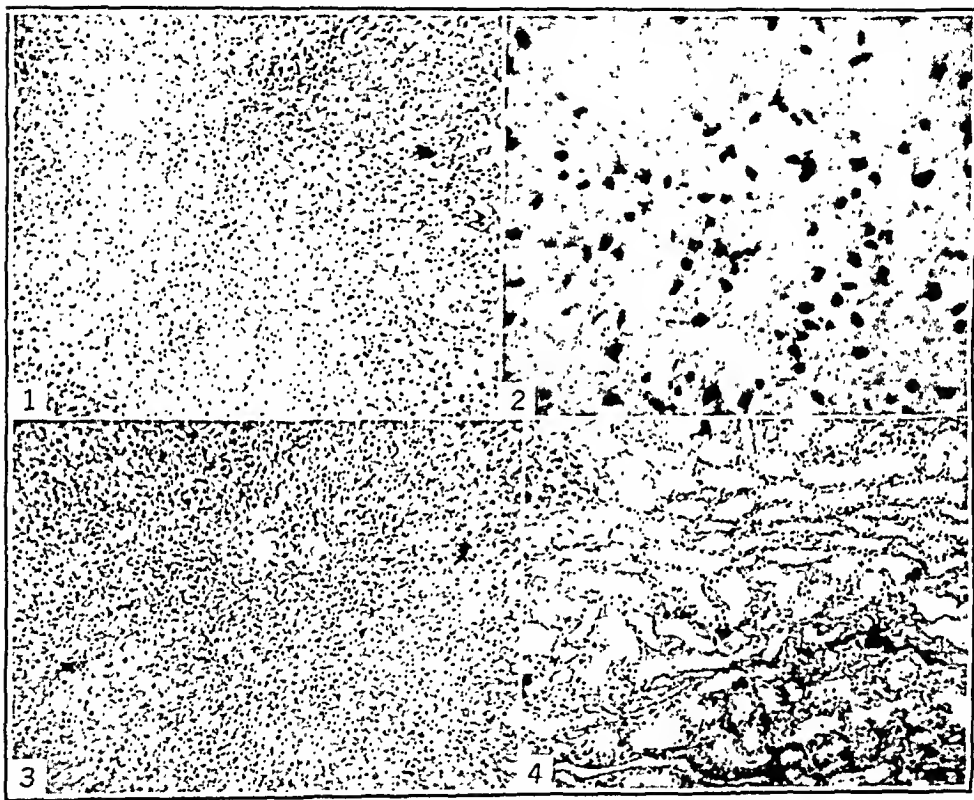


FIG. 1.—Case 1. Low-power photomicrograph of the liver demonstrating far-advanced central necrosis.  $\times 100$ .

FIG. 2.—Case 1. High-power photomicrograph of the liver showing cellular necrosis with polymorphonuclear infiltration.  $\times 440$ .

FIG. 3.—Case 2. Low-power photomicrograph of the liver showing liver cell necrosis with fatty metamorphosis in the peripheral zone.  $\times 100$ .

FIG. 4.—Case 2. Low-power photomicrograph of the kidney showing increased cellularity and avascularity of the glomerular tufts. Albuminous casts are present in the lumina of the tubules.  $\times 440$ .

**Comment.** The outstanding feature of this case is the long clinical course of 80 hours in a syndrome which ordinarily terminates in 24 hours or less. Unfortunately, no sodium, potassium and chloride determinations were obtained. Although clinically, the evidence suggested renal failure, the picture was dominated pathologically by liver necrosis (Figs. 1 and 2).

CASE 2. C. W., a 20 year old obese white male, was admitted to the hospital on Feb. 17, 1943, complaining of chills, fever, headache, and generalized muscular pains of 24 hours duration. He was seriously ill but rational and oriented. The physical examination disclosed facial edema with puffiness about the eyes. The body was covered with numerous petechial and purpuric areas. No nuchal rigidity was present. The Kernig and Brudzinski signs were absent. The spleen was not palpable. The temperature was 98.8° F., pulse 100, respirations 22, and the blood pressure 60/40. No lumbar puncture was performed due to the poor condition of the patient. A blood count revealed 66,950 white blood cells with 96% polymorphonuclears. The red cell count was 5,510,000 with a hemoglobin of 13.8 gm. A blood culture was positive for the *Neisseria intracellularis* Type I. A diagnosis of a fulminating meningococcemia with adrenal hemorrhages (Waterhouse-Friderichsen syndrome) was made.

The patient was immediately given 5.5 gm. of sodium sulfadiazine intravenously in a 5% solution of sterile distilled water. Within the first 12 hours of admission, he received a total of 80,000 units of meningococcus antitoxin intravenously. Adequate fluids were administered both orally and intravenously and 50 cc. of adrenal cortical hormone were given by the latter route daily throughout the course of the disease. Massive doses of sulfadiazine were given orally, the initial dose 8 gm. The total dose for the 1st day of therapy was 29 gm. Twenty-two hours after hospitalization, the patient received 500 cc. of plasma. The blood pressure rose to a level of 80/60. The initial blood sulfadiazine concentration 14 hours after admission was 2.5 mg.

Complete urinary suppression was observed during the first 24 hours of hospitalization. At this time the patient was catheterized and 2 ounces of cloudy, amber urine obtained. On examination, the urine was negative except for 2+ albumin. Diarrhea was a persistent complaint throughout his course. The stools were liquid, light brown in color but not bloody. No abdominal pain was present.

On the 2nd hospital day, the patient appeared restless and apprehensive but the sensorium was clear. Facial edema persisted. Temperature was 97.2° F., pulse 130, respirations 28 and the blood pressure 100/80. The patient voided 3 ounces of urine spontaneously which showed a specific gravity of 1.025 and 1+ albumin. Microscopically, a few granular and cellular casts were found with 50 to 60 red blood cells per high-power field. A blood count was 20,300 white blood cells with 95% polymorphonuclears. The non-protein nitrogen was 52 and the blood sulfadiazine level 10.2 mg. Efforts to combat the impaired renal function with hypertonic glucose, aminophylline, hot colonics, and coffee were unsuccessful.

On the 3rd hospital day, the patient was definitely worse, although still conscious and oriented. His respirations were rapid and shallow, averaging 54 per minute. Slight cyanosis was evident for the first time. Numerous new petechial and purpuric areas appeared and several older lesions showed necrotic changes. Temperature was 97.2° F., and pulse 130. The blood count was now 35,750 white blood cells with 98% polymorphonuclears. A blood sulfadiazine concentration reached a level of 11.7 mg. The non-protein nitrogen continued to rise, reaching 91 mg. per 100 cc., and the creatinin 7 mg. per 100 cc. A specimen of catheterized urine (1 ounce) showed 2+ albumin, 5 to 6 red blood cells, 1 to 2 hyaline casts, and a few cellular and granular casts. The patient was placed in an oxygen tent, because of cyanosis and dyspnea.

On the 4th hospital day, the patient lapsed into stupor and was aroused with difficulty. Weakness and cyanosis were more pronounced and air-hunger more marked. Temperature was 98° F., pulse 140, respirations 52, and the blood pressure 120/80. The blood count revealed 49,450 white blood cells with 93% polymorphonuclears. The red cell count was 4,900,000 with 11.2 gm. of hemoglobin. The blood sulfadiazine level reached 26.1 mg. The non-protein nitrogen rose to 96.9 mg. per 100 cc. and creatinin to 7.4 mg. The blood chlorides were 412.5 mg. per 100 cc.

The patient's course progressed rapidly downhill and he succumbed on Feb. 21, 1943, 88 hours following admission to the hospital.

*Autopsy.* The body was that of a well-developed, obese young man. The skin was covered with many petechial and purpuric areas, some of which showed early necrotic changes. A moderate amount of clear, straw-colored fluid (500 cc.) was present in the serous cavities. The heart was enlarged and weighed 590 gm. The right auricle showed great dilatation. The liver weighed 2790 gm. and on section showed moderate congestion. The kidneys were large and felt less firm than usual. The right kidney weighed 240 gm. and the left 280 gm. The capsule stripped with ease. On section, the kidneys showed considerable congestion. The cortico-medullary demarcations were indistinct. The brain revealed marked engorgement of its superficial vessels but no gross exudate was seen. The ventricular fluid was clear. The adrenals were hemorrhagic and on section, numerous small hemorrhages were noted involving both the medulla and cortex.

On microscopic examination, significant histologic changes were found in the adrenals, liver and kidney. The adrenals showed numerous small hemorrhages involving both medulla and cortex. The architecture of the liver lobules showed marked destructive changes in the central and mid-zones. The liver cords in these zones were completely destroyed. The cells were only occasionally recognizable and moderate bile pigmentation was present. Numerous areas of hemorrhage and polymorphonuclear infiltration were seen. In the peripheral zone, the liver cords were preserved, but the liver cells were swollen with marked vacuolization and granularity of their cytoplasm. Fatty metamorphosis was a prominent pathologic feature. The Kupffer cells were not noteworthy. The central veins and sinusoids were engorged and the portal areas frequently showed a moderate polymorphonuclear infiltration with small nests of lymphocytes. The kidney architecture was preserved. The glomeruli were normal except for albuminous deposits in the capsular spaces and marked cellularity with avascularity of the tufts. The epithelial cells of the proximal convoluted tubules showed cloudy swelling and had a "ground glass" appearance. Albuminous casts were plentiful.

**Comment.** This case is another illustration of severe meningococcemia with marked adrenal hemorrhages associated with a prolonged survival period. The clinical course and mode of death is strikingly similar to that of Case 1. Paradoxically, a sole blood chloride determination a few hours prior to death was found to be within normal range. The question is raised, therefore, as to whether the electrolyte balance is disturbed in the Waterhouse-Friderichsen syndrome. The entire clinical picture was overshadowed by impaired renal function but at necropsy, liver necrosis was an impressive feature (Fig. 3). This case is unique in that the patient survived for the amazingly long period of 88 hours despite such extensive adrenal destruction.

**Discussion.** The 2 cases of the Waterhouse-Friderichsen syndrome described in this paper were unusual because of their relatively prolonged survival periods, 80 and 88 hours respectively, and the similarity of their clinico-pathologic findings. The fulminating character of this syndrome has made impossible any detailed observations heretofore, and this appears to be the first report to describe hepatorenal failure in protracted cases of the Waterhouse-Friderichsen syndrome.

The salient clinical features of both cases were so similar as to warrant detailed discussion. The onset of the acute phase of the disease was marked by severe shock and pallor. Consciousness and orientation were maintained until shortly before death. Facial edema, particularly about the eyes, was remarkable in its similarity to that

of nephritis. Edema involving other portions of the body could not be demonstrated clinically. Interestingly enough, subnormal temperatures persisted throughout the course of the infection in spite of a severe overwhelming septicemia, and recovery from the initial shock as evidenced by a return of the blood pressure to normal or quasi-normal levels. No agonal rise in temperature occurred. Diarrhea was present from the onset and persisted throughout the course of the disease. The stools were liquid and tan in color but not grossly bloody. Jaundice or other clinical evidences of hepatic dysfunction were absent.

The striking and unusual clinical feature was the initial anuria followed by a severe oliguria which was associated with progressive impairment of renal function. This observation was noted throughout the clinical course of both patients. Although the non-protein nitrogen and creatinin rose steadily, there were no clinical manifestations of uremia.

It is important to mention at this point, our 3 other cases of the Waterhouse-Friderichsen syndrome not reported in this paper. Two of these cases had fulminating clinical courses terminating within 24 hours. At necropsy, the pertinent findings were severe hemorrhages and destruction of the adrenal glands coupled with a mild hepatitis limited to the central zones. However, no noteworthy renal changes were demonstrable. The third case survived 40 hours. In this case, hepatorenal failure was demonstrated early. The blood chemistry findings 16 hours after admission revealed a non-protein nitrogen of 46 mg. per 100 cc., creatinin 5.8 mg., and chlorides 454 mg. Thirty-six hours after admission the non-protein nitrogen rose to 67 mg. per 100 cc., creatinin 9 mg., and chlorides 470 mg. An icteric index at this time was 13.7.

On the other hand, the 2 cases of the Waterhouse-Friderichsen syndrome with prolonged clinical courses, died in a state of renal insufficiency, and showed at necropsy, moderate adrenal hemorrhages with severe hepatitis. In addition, the kidneys revealed glomerular and tubular changes and the pathologic features of both were similar but differed only in degree of severity (Fig. 4).

Case 2 demonstrated a more advanced stage of hepatic destruction than Case 1. Histologically, the noteworthy feature was severe necrobiosis of the liver cells, but whereas in Case 1 the central and mid-zones only were involved (Fig. 1), in Case 2 the central, middle and peripheral zones showed these extensive destructive changes (Fig. 3). The histologic findings in the kidneys of both cases were comparable but again differed in degree of severity. Both cases showed increased cellularity and avascularity of the glomerular tufts. Similarly, cloudy swelling of the convoluted tubular epithelium and albuminous deposits in the lumina were present but more marked in Case 2 (Fig. 4). These findings were essentially in agreement with those described by Wilensky<sup>23</sup> in the hepatorenal syndrome. From the above facts, life expectancy appears to be inversely proportional to the extent of adrenal hemorrhage; that is, the greater the adrenal hemorrhage, the shorter

the clinical course and the less likelihood for the development of hepatic destruction.

It seems logical to conclude from a correlation of the clinico-pathologic evidence in our cases that 2 clinical stages exist in the Waterhouse-Friderichsen syndrome. The first or fulminating stage is generally recognized and consists of severe shock with circulatory collapse. Survival of this stage ushers in the hitherto undescribed second or hepatorenal stage which is characterized clinically by marked oliguria with azotemia and pathologically by severe toxic hepatitis associated with glomerular and degenerative tubular changes.

The problem of why hepatorenal failure occurred only in the cases of the Waterhouse-Friderichsen syndrome and not in uncomplicated meningococcemias, cannot be ignored. Infection alone fails to explain satisfactorily this puzzling fact and our experience has shown that the hepatorenal syndrome does not occur in meningococcemia without adrenal involvement. It must be concluded that adrenal hemorrhage is a predisposing factor in the development of hepatorenal failure in the Waterhouse-Friderichsen syndrome. After evaluating the existing theories on the hepatorenal syndrome, coupled with an analyses of our cases and stressing the important rôle played by the adrenals in detoxification and its close interrelationship with liver and kidney function, it seems reasonable to advance the following explanation for the mechanism producing hepatorenal failure in the Waterhouse-Friderichsen syndrome. Destruction of the adrenal glands by hemorrhage throws the full brunt of detoxification primarily upon the liver and secondarily, upon the kidneys, both of whose functions have been depressed from the onset by shock and toxemia. The partially disabled liver is so overwhelmed by toxins that it is unable to perform its physiologic detoxifying function. As a result, the liver undergoes toxic necrosis and a potent tissue toxin is elaborated which, together with the unneutralized bacterial toxins, flood the circulatory system. Finally, the kidneys attempt to rid the body of these harmful products of bacteria and deranged liver metabolism by excreting them through the proximal convoluted tubules. The renal tubules, in the performance of this function, undergo degenerative changes. In addition, glomerular changes of a nephritic type occur. The avascularity of the glomerular tufts is difficult to evaluate but we believe it is indicative of diminished renal blood flow. All these factors operating in unison plausibly explain the renal failure.

A fact worthy of consideration is that the toxins elaborated by the necrotic hepatic cells are harmful to the comparatively uninvolved normal tissue remaining in the liver. This was proven experimentally by Boyce<sup>2</sup> who reproduced similar pathologic changes in dogs by the intraperitoneal injection of water-soluble extracts of liver tissue from patients dying of the hepatorenal syndrome. The changes produced were lethal or sublethal depending upon the strength of the extracts employed.

In spite of the vigorous use of accepted methods of therapy, the out-

come of our cases was invariably fatal. The therapeutic measures employed may have contributed in prolonging the clinical course. However, when all factors are considered, it seems inconceivable that a patient can recover from the Waterhouse-Friderichsen syndrome. To support this contention is the unassailable evidence of severe crippling of hepatorenal function as has been pointed out and its utter incompatibility with life can hardly be questioned.

**Summary.** Two cases of the Waterhouse-Friderichsen syndrome with prolonged survival periods of 80 and 88 hours respectively and with death ascribed to hepatorenal failure are presented in detail. This appears to be the first such report in the literature. These cases are unique in that they are characterized clinically by renal failure and pathologically by hepatic necrosis and glomerular and tubular changes in the kidney. These findings are similar to those found in the hepatorenal syndrome.

In the light of our clinical observations, it is necessary to conclude that 2 stages exist in the Waterhouse-Friderichsen syndrome. The first stage is a fulminating one usually resulting in death from shock and toxemia. Survival of this period ushers in the second or hepatorenal stage wherein death ensues from hepatorenal failure.

The life expectancy in our cases was inversely proportional to the degree of adrenal tissue destroyed by hemorrhage.

Accepted modes of therapy administered to our patients may have prolonged the clinical course but did not alter the fatal outcome of the disease.

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# PROGRESS OF MEDICAL SCIENCE

## PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

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### OBSTRUCTIVE LESIONS OF THE MAIN RENAL ARTERY IN RELATION TO HYPERTENSION

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DURING the past decade a spotlight of considerable intensity has been focused upon the problem of experimental hypertension. A recent review of the present status of the relationship between human hypertension and that produced experimentally<sup>30</sup> indicates that although many questions pertaining to hypertension, as it occurs in human beings, have been clarified, others of a fundamental nature still remain obscure. There appears to be a widespread, tacit acceptance of the idea that an etiologic association exists between hypertension produced experimentally and that occurring in a great variety of pathologic lesions of the kidneys, notably those which affect the renal blood-vessels. Such an assumption implies that a similar mechanism acts to produce the hypertension in each instance. While the final proof of such a relationship must naturally rest upon an exact correlation of pathologic lesions with experimental methods, this is not always possible to make.

Diffuse sclerosis or constriction of intrarenal arteries has proved, up to the present time, to be technically impossible to produce in experimental animals as a means of initiating hypertension. Therefore, direct comparison cannot be made between experimental hypertension as produced by present methods and hypertension in man associated with chronic glomerulonephritis, chronic pyelonephritis or nephrosclerosis in all of which the vascular lesions occur in intrarenal arteries. On the other hand, lesions causing constriction of one or both main renal arteries closely simulate the experimental conditions of the arterial clamp used so successfully by Goldblatt,<sup>11</sup> Wilson and Byrom,<sup>31</sup> and others and provide probably the only human examples of hypertension in which the various factors can be accurately evaluated and in which a direct comparison with experimental procedures can be made. The present review comprises a critical survey of reported data bearing on the fundamental question of the rela-

tion of obstructive lesions of the main renal artery to hypertension in man. It is hoped that a clear understanding of the established facts pointing to a relationship between human and experimental hypertension will make the problem stand out in sharper relief and act in some measure as a guide to further investigations.

The literature of recent years contains a number of case reports and statistical surveys purporting to show that the "Goldblatt mechanism" is active in at least some cases of human hypertension, but despite the interpretations of most of the authors, such proof as does exist rests largely on circumstantial evidence. The elusive character of the problem depends, in the main, on the almost unavoidable failure to reproduce in any given human case all the steps required for a clear-cut, conclusive experiment.

It is natural that interest should have centered largely, but not exclusively, on examples of unilateral renal disease, reviewed recently by Abeshouse,<sup>1</sup> in which there is a possibility of removing the suspected cause of the hypertension by nephrectomy. This phase of the problem received an added impetus following the demonstration by Wilson and Byrom<sup>31</sup> that, in the rat, a variety of unilateral renal disturbances, including arterial constriction, would cause a persistent elevation of blood pressure. That man's reaction under these circumstances is probably more like that of the rat than of other experimental animals is indicated by the relatively few instances in which nephrectomy for a unilateral lesion constricting the main renal artery has successfully lowered the blood pressure from hypertensive levels. Unfortunately, the majority of reported cases have not been clarified until after death and, although much suggestive evidence has been derived from postmortem reconstruction, it is hoped that in the future more stress will be laid upon accurate diagnosis, early treatment and adequate follow-up studies.

Occlusive lesions of the renal arteries may be roughly divided into those which narrow the lumen from within and those in which the constriction is due to extrinsic factors. The available data will, therefore, be discussed under these 2 headings:

**Narrowing Due to Intrinsic Factors. ARTERIOSCLEROTIC PLAQUES.** An arteriosclerotic plaque, at or near the orifice of the vessel, would appear to be the commonest cause of unilateral or bilateral constriction of the renal artery, whether associated with hypertension or not. A number of individual cases of this type have been reported in the literature in which constriction of one or both main renal arteries by arteriosclerotic plaques has been assumed to bear an etiologic relationship to the hypertension which was present. In none of these is the evidence absolutely conclusive, although the vascular stenosis may have been a factor.

Freeman and Hartley<sup>10</sup> recorded the case of a 57 year old man whose right kidney was removed 2 years before death following traumatic rupture. Eight months before death his blood pressure was found to be 240/140, and at autopsy a large arteriosclerotic plaque was found narrowing the orifice of the left renal artery. The left kidney weighed 200 gm. and showed no arteriolar sclerosis, but there was some focal scarring and an interstitial inflammatory reaction. This case would appear to be a fairly clear-cut example of the Goldblatt mechanism; but there is a definite possibility of hypertension developing after nephrectomy in the absence of any such specific lesion, particularly in a man of this age.<sup>5,14</sup>

Another case of hypertension associated with a solitary kidney supplied by an obstructed renal artery in a man of 56 is that of Riggs and Satterthwaite.<sup>25</sup> The history indicated a sudden onset of severe hypertension

about 1 year before death, followed by rapid loss of vision 6 months later. Terminally the blood pressure was 260/140 and death occurred in uremia. Congenital absence of the left kidney, ureter and renal artery was discovered at autopsy, and the single remaining kidney showed marked sclerosis of intrarenal vessels, atrophy of glomeruli and tubules, and slight arteriolar necrosis. There was partial obstruction of the right renal artery due to a small, localized dissecting aneurysm and an arteriosclerotic plaque at the mouth of the vessel. In addition, the branches of the renal artery entered the kidney at a sharp angle. Any or all of the above disturbances could have caused renal ischemia, and the presence of marked vascular changes within the kidney is an added complication. This case illustrates some of the problems encountered in interpreting the relationship between hypertension and narrowing of the main renal artery.

In the case reported by Stewart<sup>28</sup>, a 37 year old man with hypertension of less than 3 years duration who died from uremia with cardiac decompensation and anemia was found to have the orifices of both renal arteries surrounded and markedly stenosed by arteriosclerotic plaques. Both kidneys were considerably reduced in size, weighing 75 and 90 gm., and showed no arteriolar sclerosis but widespread destruction of parenchyma with fibrosis and lymphocytic infiltration. The changes were interpreted as the result of ischemic atrophy, and the view was supported by the fact that an aberrant artery apparently supplied additional blood to the least affected portions of each kidney. Although the author's interpretation of this case, that reduction in size of the orifices of the renal arteries resulted in a sufficient degree of ischemia to produce atrophy of the kidneys, is a tempting one, it is difficult, from the clinical and pathologic findings, to exclude some obscure intrarenal lesion as the initiating factor.

Finally such cases of arteriosclerotic occlusion of the main renal arteries associated with hypertension as those presented by Leiter (Case 2),<sup>16</sup> Saphir and Ballinger (Case 3),<sup>27</sup> and Tomlinson<sup>29</sup> hardly warrant inclusion in this review, since in each instance widespread changes in the renal parenchyma, sufficient in themselves to have caused the hypertension, were present in addition to the occlusive lesion in the renal arteries.

Seeing in these lesions a probable counterpart of the renal artery clamp used in the production of experimental hypertension, various investigators have endeavored to test the validity of the Goldblatt mechanism in humans by a statistical study of the occurrence of arteriosclerotic narrowing of the renal artery or arteries in large groups of autopsied cases of hypertensive and normotensive individuals.

One of the early references to renal arteriosclerosis as a possible factor in the production of hypertension was made by Moritz and Oldt.<sup>21</sup> These authors, in a study of arteriolar disease in a series of 100 hypertensive and 100 non-hypertensive individuals, found only "three cases of chronic hypertension in which there was no significant degree of renal arteriolar sclerosis." In all 3 cases arteriosclerosis of the main renal arteries was thought to be of sufficient severity to account for a decrease in the blood flow through the kidneys. Detailed descriptions of the vessels were not reported, however, and no mention was made of the state of the renal arteries in the other 97 hypertensive cases nor in the control group.

In 1939 Oppenheimer, Klemperer and Mosehkowitz<sup>22</sup> reported 18 cases of unilateral renal artery constriction, presumably due to arteriosclerosis, from a series of 5000 consecutive autopsies performed at the Mount Sinai Hospital, New York City. Of this group 83% had hypertension, but in every instance there was evidence of definite intrarenal arterio- and

arteriolar sclerosis with arterial narrowing on the affected side which, at times, was even more marked than in the opposite kidney. From this finding and the associated severe arteriosclerosis of the aorta in these cases and controls with hypertension, it was concluded that the renal arterial constriction was an effect rather than a cause of the hypertension. Also in 1939 Blackman<sup>2</sup> found arteriosclerotic narrowing of one or both main renal arteries in 86% of 50 cases of essential hypertension as opposed to a comparable degree of arteriosclerosis and stenosis in only 10% of a control group of 50 non-hypertensive cases. Changes in the intrarenal arteries were present in every instance. There was a much lower incidence of acute arteriolar lesions in kidneys of which the main renal arteries were markedly narrowed. This suggests that the occlusive lesions exert a protective action on the blood-vessels of the affected kidneys in the terminal phase of the disease, but no light is thrown on the question of their etiologic relationship to the hypertension.

Essentially similar results have been more recently reported by Richardson.<sup>24</sup> In a series of 145 almost consecutive autopsies, 32 cases of essential hypertension were encountered. Of these, 25 showed the presence of arteriosclerotic plaques causing varying grades of stenosis of one or both main renal arteries. This finding contrasted sharply with that in 113 non-hypertensive controls where comparable lesions in the main renal arteries were seen in only 3 cases. As in the other series reviewed, arteriolar sclerosis was found in the kidneys of practically all the hypertensive group, irrespective of the presence or absence of stenosis of the main renal artery. Richardson attempted to reconcile this occurrence, with the constant absence of such intrarenal sclerosis in experimental hypertension due to clamping the renal arteries, by suggesting that any protective action occurs only in the late stages of the plaque development when stenosis is extreme, whereas blood pressure increases with the gradually increasing ischemia.

The results of the most recent study of the relationship between arteriosclerosis of the renal artery and hypertension, by Lisa, Eekstein and Solomon,<sup>17</sup> differ markedly from those of the authors already quoted. In 100 consecutive cases coming to autopsy in which blood pressure readings were available, hypertension was present in 56, while 44 were non-hypertensive. No appreciable difference in the average diameter of the renal arteries was found in the 2 groups, and in only 2 instances was there any marked stenosis due to arteriosclerotic plaques. Of interest also in this series was the large number of non-sclerotic renal vessels found in the hypertensive group. In 40% of these individuals, the vessels were regarded as normal while arteriosclerosis was absent in 56.8% of the non-hypertensives. Another significant finding was that little, if any, correlation could be demonstrated between the amount of sclerosis and the degree of constriction of the renal arteries.

Conclusions in the studies of Blackman<sup>2</sup> and Richardson<sup>24</sup> were based on similar methods of measuring the lumen of the renal arteries. This involved sectioning the vessels after fixation and estimating the degree of stenosis. In Blackman's report, actual measurements were made from stained microscopic sections. Lisa *et al.*, on the other hand made measurements of the arterial lumina in the unfixed state with graduated sounds and compared these with the size of the arteries measured according to Blackman's method. There was no constant relation between the size of the vessels when measured by these 2 methods and many non-sclerotic vessels with a good calibre in the unfixed state showed a considerable degree of narrowing after fixation and staining. On the basis of these

findings, it would seem that attempts to correlate the degree of narrowing of the lumen which existed during life with that observed in fixed, stained sections are of very questionable value.

From the above reports, it is apparent that atheromatous plaques in the renal arteries, usually associated with severe atherosclerosis in the aorta and elsewhere, occur in a high percentage of individuals with "essential" hypertension. However, critical analysis of the evidence would seem to favor the view that these lesions are secondary or incidental to the hypertension rather than causative in all but the few instances in which renal arteriolar sclerosis is absent.

**EMBOLISM AND THROMBOSIS.** The association of hypertension with partial or complete occlusion of the main renal arteries resulting from thrombosis or embolism has received relatively little attention in the past. However, a study of lesions of this type may well prove to be most fruitful in attempting to solve some of the still unanswered problems concerning the relationship between experimental and human hypertension.

It is often difficult, or even impossible, to ascertain definitely in this type of case whether the occlusion is due to embolism or thrombosis *in situ* so that the two will be considered together. The literature contains very few examples of thrombosis of the main renal artery associated with hypertension, and it is only within the past few years that hypertension and renal artery embolism with infarction have been linked together. In fact, it has frequently been stated that renal infarction is not followed by an elevation of blood pressure.

The often quoted case of Boyd and Lewis<sup>4</sup> in which relief from hypertension resulted after the removal of a partially infarcted kidney possibly belongs in this group, but cannot be seriously considered because of the lack of specific pathologic details and the suggestion by the authors that widespread vascular changes throughout the kidney had greater significance than the infarct.

Four cases of hypertension due to renal embolism have been reported by Fishberg.<sup>9</sup> In the 1st of these a transient rise in blood pressure of 4 days duration occurred following events which led to a clinical diagnosis of infarction of the left kidney. The 3 other individuals, each suffering from mitral stenosis, developed hypertension which persisted until death, at variable periods after the occurrence of renal artery embolism. On postmortem examination complete occlusion of the main trunk of the right renal artery with massive but not complete infarction of the kidney was observed in 2 instances. No excretion of dye took place on the affected side following intravenous pyelography nor was any urine obtained by ureteral catheter in 1 of these cases. In the 3rd case, the only one in which renal failure developed, the left main trunk and the right middle branch of the renal arteries were involved.

Prinzmetal, Hiatt and Tragerman<sup>23</sup> made a careful study of a patient with chronic rheumatic heart disease who developed hypertension several days after an attack of abdominal pain. Death occurred in uremia 3 weeks later, during which time the elevation of blood pressure persisted while there was progressive suppression of urine and retention of nitrogen. An autopsy revealed thrombotic occlusion of both main renal arteries, probably embolic in origin, and extensive though incomplete bilateral infarction of the kidneys. Perfusates of 1 kidney contained a pressor substance similar to that found in perfusates of ischemic kidneys of animals. In this case the hypertension was presumed to be due to the same mechanism as that responsible for the hypertension following the termination of complete renal ischemia in experimental animals.

From the clinical standpoint, thrombosis of the renal artery has not been stressed until recently. Wolfe,<sup>32</sup> in a report confined almost exclusively to immediate clinical manifestations, discussed 27 cases in which a diagnosis of thrombosis of the main renal artery or its branches was made. The criteria on which the diagnosis was based are not too firmly established since in only 1 instance was postmortem confirmation obtained. However, it is interesting to note that a sudden elevation of blood pressure occurred in a high percentage of the more severe cases.

Saphir and Ballinger<sup>27</sup> were the first to present a detailed report of hypertension associated with thrombosis of 1 main renal artery (Case 1). It concerned a 46 year old male in whom arterial hypertension was first noted 3 years after a fracture of the pelvis. Two years later following an automobile accident, a retroperitoneal mass was discovered in the left upper quadrant. Death occurred in uremia about 9 months later following the development of congestive heart failure, and while the patient was under observation in hospital the blood pressure readings varied from 205/100 to 254/120. Pyelography showed excretory function to be excellent, and the left kidney was depressed by an extrinsic mass. At autopsy the left renal artery was smaller than the right, the orifice was markedly stenosed, and the lumen was almost completely occluded by old organized, partially calcified, thrombus material and some more recent thrombus. The left kidney revealed no abnormality other than a small area of infarction, whereas the right kidney with a widely patent artery presented a typical picture of malignant nephrosclerosis. The mass palpated clinically proved to be an organized hematoma of the left adrenal gland. There can be little doubt that a causal relationship existed between the constriction of the left renal artery and the hypertension. However, the exact sequence of events and the rôle of the thrombosis, exclusive of the other stenosing factors in producing the ischemia, would seem to be matters for speculation.

Two additional cases of unilateral renal artery thrombosis with hypertension, observed by the Reviewer within a period of 5 months, and at present being prepared for publication, will be briefly summarized:

The first patient, a healthy 59 year old male, with no previous complaints referable to his terminal illness, was found to have a systolic blood pressure of between 180 and 190 mm. of mercury 5 weeks after the last of a series of routine periodic examinations. Previous blood pressure readings taken on numerous occasions had averaged 140/80. Hypertension persisted, despite intermittent red rest, over a period of 3 months after which time the patient entered hospital for investigation. Physical examination was essentially negative except for a chronic pansinusitis and a blood pressure of 192/122. A careful study of the genito-urinary tract revealed a small non-functioning right kidney, and a large left kidney with normal function. There was no retention of nitrogen, but the urea clearance was diminished to about 50% of normal. The blood pressure remained at high levels which ranged between 180 to 220 systolic and 120 to 140 diastolic with occasional peaks as high as 250/150 mm. of mercury. Approximately 5 months after the hypertension had been discovered, a right nephrectomy was performed. At operation, which had been delayed for several weeks on account of a troublesome sore throat, no pulsations could be felt in the right renal artery. Postoperatively the blood pressure fell within a few hours to 140/60 and on the following day became stabilized at about 90/60. The patient developed pneumonia and died 40 hours after the operation.

The right kidney, removed surgically, weighed 134 gm. and showed no infarction or other gross abnormalities. Microscopically there was a conspicuous absence of vascular sclerosis affecting either arterioles or arteries. At autopsy the right renal artery, which had been ligated close to the kidney, was occluded near the aorta by a mass of thrombus material 6 mm. in length. The central part of this thrombus was organized and recanalized while at either end it appeared more recent and was undergoing organization. The abdominal aorta was the seat of marked arteriosclerosis but in both renal arteries atheromatous changes were minimal. The left kidney weighed 255 gm. and the histologic appearance was that of extreme hypertrophy. In this, the unobstructed kidney and in other organs, notably pancreas, adrenals and liver, there was a diffuse hyaline thickening of small arterioles. The heart was hypertrophied and there was a widespread, early, acute bronchopneumonia in both lungs.

It is impossible to determine whether the arterial occlusion resulted from thrombosis *in situ* or embolism from a mural thrombus in the abdominal aorta, but since the age of the thrombus in the right renal artery could readily approximate the 5 months duration of the hypertension, it seems reasonable to assume that occlusion of this vessel with the resulting unilateral ischemia initiated the sudden elevation in blood pressure.

The second case is that of a 55 year old male who was admitted to hospital with a diagnosis of hemorrhage from a gastric ulcer. There was no previous history of hypertension, but despite a considerable degree of exsanguination over a period of 2½ weeks the blood pressure readings averaged 150/90. During this time transfusions and conservative treatment failed to cause improvement and the hemorrhage was finally arrested by a widespread ligation of blood-vessels supplying the lesser curvature of the stomach. Shortly after this operation the blood pressure rose to 200/100 and remained at consistently high levels up to 260/150. The patient had a convulsive seizure on the second postoperative day. Following this, during a period of improvement, an intravenous pyelogram failed to show any excretion of dye on the left side and retrograde studies revealed a small left kidney without appreciable function but with a normal pelvis and ureter. Renal function was fairly good as indicated by a 40% excretion of phenol sulphonphthalein in 2 hours and a blood non-protein nitrogen level of 32.8 mg. per 100 cc. These studies suggested that an anomalous left kidney might be the cause of the hypertension and left nephrectomy was planned. However, before this could be carried out, the patient had a second generalized convulsion followed by death 2 weeks later and 6 weeks after the gastric operation.

At autopsy the main branch of the left renal artery was completely occluded near its orifice, by a relatively recent, organizing thrombus. This blocked the lumen for a distance of approximately 1 cm., had occurred over an area of moderate arteriosclerosis and appeared to have been present for at least 4 to 6 weeks. The lower abdominal portion of the aorta showed marked arteriosclerosis. The left kidney was atrophic, weighing only 75 gm. and contained a recent infarct at the upper pole posteriorly, related to a small, somewhat narrowed, extrarenal branch of the renal artery. Microscopically, the non-infarcted tissue appeared slightly condensed but otherwise normal, and there was no suggestion of arteriolar sclerosis. The right kidney was relatively hypertrophied, weighing 180 gm. and on histologic examination, numerous thickened, hyaline arterioles were encountered. The heart was not appreciably hypertrophied.

There is little reason to doubt that the hypertension, at least at its

terminal level, was related to the profound circulatory disturbances in the left kidney. The postmortem findings do not suggest that either the hypertension or the renal artery thrombosis had been present before the terminal illness, and it is quite possible that a sudden onset of hypertension shortly before admission to hospital precipitated the gastric hemorrhage. On the other hand, the occurrence of thrombosis or embolism at the time of operation cannot be entirely excluded.

**CONGENITAL LESION.** The well known and frequently quoted case of Leadbetter and Burkland<sup>15</sup> still remains the most clear-cut and possibly the only convincing human counterpart of the "Goldblatt phenomenon" as seen in experimental animals. In this instance a colored boy was found to have an enlarged heart at the age of 6 months. Hypertension was definitely established when the child was 3 years old and the first blood pressure determinations were made. Further studies at this time disclosed an ectopic right kidney. No relationship between the hypertension and the ectopic kidney was considered until 2 years later when nephrectomy was performed. The lumen of the right renal artery was found to be almost completely occluded by a mass of smooth muscle, a lesion which was probably congenital in origin. The blood pressure fell promptly after operation to a relatively normal level and a subsequent report published 3½ years later stated that there had been no return of the hypertension.<sup>6</sup> At this time the child, aged 9 years, was healthy and active with a blood pressure stabilized at about 105/65 mm. Hg.

**Narrowing Due to Extrinsic Factors. KINKING AND TORSION.** Obstruction of the renal artery due to rotation and twisting of the pedicle of a movable kidney should theoretically correspond in some measure to intermittent clamping of the renal artery in experimental animals; but orthostatic hypertension is apparently not very frequently associated with nephroptosis. However, McCann and Romansky<sup>20</sup> demonstrated quite clearly, in a small group of cases with nephroptosis, that the blood pressure was elevated and that the total renal blood flow was diminished in the erect posture as compared with the findings when the individuals were recumbent. Their postulation of a transient interference with the blood supply of the kidney resulting in increased renin production, under these conditions, seems quite reasonable, although attempted fixation of the kidney by nephropexy or the application of a belt did not appreciably reduce the blood pressure.

Riskind and Greene<sup>26</sup> subsequently reported the case of a 47 year old woman, suffering from menopausal symptoms, whose blood pressure over a 2 year period ranged between 170/100 and 220/120. Complete bed rest in the horizontal position lowered the blood pressure to some extent and retrograde pyelography revealed no abnormalities in the recumbent position. However, in the upright position a 45 to 60 degree torsion of the pelvis on the right side was noted. The patient's course was reported for 1 year after the application of a corset, during which time a relatively normal blood pressure was maintained except for occasional periods when high levels were recorded. These were usually associated with removal of the corset.

The relatively scant data in this field suggest a definite relationship between the hypertension and disturbances in renal blood supply, but the rôle played by such factors as obstruction to the venous return from the kidneys and to the outflow of urine needs further elucidation.

**ANEURYSM OF RENAL ARTERY.** Aneurysms of the renal artery; although questionably producing occlusive lesions, will be briefly discussed. Howard,



Forbes and Lipseomb<sup>13</sup> were perhaps the first to draw attention to the possible relationship between this relatively uncommon condition and hypertension. They reported the case of a 5 year old girl whose blood pressure was known to be within normal limits 6 months before the occurrence of a small cerebral hemorrhage in the right frontal region. The blood pressure at this time ranged between 158/90 and 200/140. Hypertension persisted and retrograde pyelography on 2 occasions revealed no abnormality. The urine contained a few casts and a trace of albumin. A right lumbar sympathectomy had no effect on the blood pressure and 2 weeks later during a sympathectomy on the left side, a thin-walled, multilocular, pulsating mass was found attached to the left renal artery. The vessel was ligated through the mass and the left kidney was removed. The blood pressure which was 170/100 before nephrectomy fell within 5 minutes to 140/90 and remained at slightly elevated levels in the neighborhood of 125/90 for 9½ months. Interpretation of this case is extremely complicated, since a possible effect of the bilateral sympathectomy cannot be excluded, and pathologic examination of the kidney removed at operation revealed subchronic glomerulonephritis. This was presumably related to an attack of acute hemorrhagic nephritis diagnosed when the child was 2 years of age and could readily account for the postoperative persistence of a slight elevation of blood pressure and moderately lowered urea clearance.

All previously reported cases of aneurysm of the renal artery were reviewed in 1932 by Mathé<sup>19</sup> and the review was brought up to date in 1943 by Lowsley and Cannon.<sup>18</sup> In only 4 of the first group of 56 cases was hypertension noted while 2 others showed cardiac hypertrophy. In the later series, hypertension was present in 4 out of 21 cases and was apparently cured by nephrectomy in 1 instance. It is, of course, quite possible that an aneurysm in this location could alter pulsation within the kidney or cause ischemia by direct pressure on the renal pedicle, but available data provide little more than a suggestion of an occasional association between this type of lesion and hypertension.

**EXTERNAL PRESSURE.** Reported cases of hypertension associated with constriction of one or both renal arteries by pressure from without are few in number and the actual frequency of such an association is probably not accurately reflected in the literature. The 4 cases reviewed below illustrate the pressure effects of widely different lesions. They are pertinent to the present discussion, because each is cited by the respective authors as an example of the operation, in man, of the Goldblatt mechanism of the production of hypertension.

In the case of Blatt and Page<sup>3</sup> a male aged 38 years died about 11 months after the onset of symptoms referable to a widespread lymphosarcoma. Early in the course of the disease he developed a persistent hypertension and at autopsy a large mass of tumor tissue was found enveloping the kidneys, renal vessels, ureters, abdominal aorta, inferior vena cava and adrenal glands. Some evidence of early glomerulonephritis was also found on histologic examination of the kidneys. Although fully aware of the numerous possible factors in this case that might have been responsible for the development of hypertension, the authors stress partial constriction of the renal arteries as the most important single factor.

The association of a large hydatid cyst of one kidney with a clinical picture of malignant hypertension and the typical changes of malignant nephrosclerosis in the opposite kidney was reported by Davson.<sup>7</sup> Although partial occlusion of moderately large branches of the renal artery was demonstrated at autopsy, the simultaneous occurrence of dilatation of the

renal pelvis with compression and fibrosis of kidney parenchyma makes an exact reconstruction of the mechanism of disturbed blood supply impossible in this instance.

A more clear-cut example of hypertension related to external pressure on one renal artery is found in the case described by Hoffman.<sup>12</sup> This concerned a 28 year old negro with syphilis and hypertensive heart disease. Death occurred about 1 year after the onset of symptoms, and a post-mortem examination revealed a saccular aneurysm of the aorta, 6 cm. in diameter, which was wedged between the aorta and the left kidney, causing marked compression of the renal artery and vein. The kidney with the disturbed blood supply was atrophic, whereas the opposite kidney was hypertrophied.

In contrast to the 3 previous fatal cases, the last to be described, reported by Farrell and Young,<sup>8</sup> was apparently cured by nephrectomy. An 18 year old male was found on routine examination to have a blood pressure of 154/102 mm. of Hg and physical examination revealed a large mass in the region of the right kidney. An intravenous pyelogram showed no abnormality but on retrograde pyelography distortion and displacement of the right renal pelvis was noted. The right kidney, removed at operation, was encased in a large cystic structure, diagnosed pathologically as an old organized and partially calcified hematoma, which probably resulted from an injury incurred when the patient was 6 years of age. Two thickened and narrowed renal arteries entered the kidney through the wall of the cyst which presumably also surrounded the renal veins. Histologic changes in the kidney were minimal. The mechanism of production of the hypertension in this case was thought to be similar to that in experimental cellophane perinephritis, but it is difficult to exclude the effects of compression of the extrarenal blood-vessels.

In all instances of extrinsic pressure on the pedicle of the kidney, whether associated with hypertension or not, venous or ureteral compression is a distinct possibility, if not an actuality, so that the specific effect of arterial occlusion cannot be clearly evaluated.

**Summary and Conclusions.** The actual number of reported cases in which hypertension is associated with obstructive lesions of one or both main renal arteries in man is not large and in only a small percentage of these can a definite etiologic relationship be established between the vascular lesion and the elevation of blood pressure. Most of the material reviewed consists of fatal cases in which attempts have been made to correlate postmortem findings with hypertension observed before death. A great deal of indirect, but highly suggestive evidence, in favor of an etiologic association between occlusive lesions of the main renal arteries and hypertension, is supplied by this group of cases, although in many instances exact interpretations are rendered difficult or impossible by the coexistence of other intrarenal or extrarenal lesions, possibly responsible for the initiation or accentuation of hypertension. In the face of such complicating factors there has perhaps been too great a tendency to assume the action of the "Goldblatt mechanism" merely because of the presence of a lesion which narrowed the lumen of one or both main renal arteries. On the other hand, it is noteworthy that no evidence, opposed to the view that experimentally produced hypertension has a human counterpart, was encountered. From the findings in occasional specific instances, benefited by nephrectomy, it can be reasonably concluded that a mechanism, similar to that responsible for the production of hypertension following the application of a clamp to the main renal artery in

experimental animals, is capable of acting in the human subject. Despite the relatively few reported cases in which occlusion of the main renal artery apparently played a rôle in the production of hypertension, the wide variety of lesions capable of causing arterial obstruction suggests that the action of this mechanism may occur more frequently than is indicated by the literature.

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## PREVENTIVE MEDICINE AND EPIDEMIOLOGY

UNDER THE CHARGE OF

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## TROPICAL DISEASE AND GLOBAL WAR

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**Disease in War Time.** Prior to December 7, 1941, few Americans were concerned with the possibility of the entrance of exotic diseases into the continental United States. Public health<sup>35</sup> and military officials,<sup>30</sup> however, have been deeply concerned for many years, fearful of the extension of the plagues of war to this continent. Although war is often limited by political boundaries, disease recognizes no such limitations. Bacteria do not recognize any law and are equally dangerous to friend and foe.

Since the beginning of history, wars<sup>41</sup> have inevitably been attended by pestilence and disease. Throughout the records of man,<sup>28</sup> famine and

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disease are constant companions of war. Their goal is death and destruction. The outcome of campaigns and the course of history have, upon occasion, been determined not by military operations but rather by disease outbreaks and epidemics. Nations have been defeated by war-brought pestilence. Armies<sup>40</sup> have been decimated by malaria, smallpox and plague. European history is, in fact, full of accounts of "French" disease, "Spanish" disease and others where armies have been traveling reservoirs of infection, spreading both endemic and epidemic disease throughout the area which they invaded. The resulting outbreaks have, however, in the main been restricted to zones of military operations and only rarely followed the soldier on his return home from distant war fields.

Prior to the 20th century,<sup>6</sup> wars were characterized by great epidemics of typhus fever, smallpox, typhoid fever, dysentery, malaria, cholera and bubonic plague. In 20th century warfare, influenza, meningococcus meningitis and other respiratory and arthropodal-borne diseases are the chief concern of the military and civilian health authorities. Under war conditions,<sup>13</sup> many of the safeguards of the health of both military and civilian populations are abandoned. The intimate contact between individuals who are herded into concentration or military camps, or who are crowded into bombshelters, or into confined quarters in an effort to keep warm, provides ample opportunity for the transfer of exoparasites or direct infection. Under such primitive environmental sanitation and inadequate personal hygiene, conditions for the spread of infections are ideal. This concentration and movement of armies and refugees, accompanied by the hardships of exposure and fatigue, malnutrition and the lack of proper medical care, provide the fuse for explosion of widespread epidemics.

More soldiers were killed by infectious disease than by bullets in all wars prior to the Franco-Prussian War of 1870, at which time the German armies reported more deaths from battle than from disease. In the Russo-Japanese War of 1904, both sides experienced more disability from war casualties than from infectious disease. Our own A.E.F. of 1917 and 1918,<sup>8</sup> in spite of the toll of influenza, reported fewer deaths from sickness than from battle. If, however, the data for the entire United States Army, at home and abroad, are taken into account, the deaths from disease in those years were more numerous than those from battle casualties.

WORLD WAR I. During and after World War I, all infectious diseases normally prevalent, except the common childhood diseases, were highly prevalent in eastern Europe. There was an epidemic of typhus fever and relapsing fever through eastern Europe, especially Russia, Poland and Siberia. Fortunately, these diseases did not spread into western Europe and to regions where the American army was fighting. Overshadowing all other epidemics was the influenza pandemic which caused an increased fatality from pneumonia. Meningococcus meningitis was epidemic, while dysentery, smallpox, tuberculosis and venereal disease increased in prevalence. Since the vast portion of the fighting in World War I occurred in continental Europe, tropical disease played a relatively minor rôle except for malaria, which became highly epidemic in certain regions of southern Europe. Malaria<sup>18</sup> was victor in Macedonia and decimated the opposing armies to a pathetic ineffectual fraction. Although it had previously disappeared from most of Europe,<sup>5</sup> malaria returned with the soldiers and refugees to central and northern Europe to ravage the civilian population for over a decade. The United States, fortunately, escaped the importation of tropical diseases which were stirred up in that conflict. Since our armies did not fight in the areas where these diseases were prevalent, they did not succumb to them; nor did they bring them back upon their return.

**THE PRESENT WAR.** This global war<sup>31</sup> is much more hazardous to civilian populations than any in history. Zones of intensive military activity extend to all continents, into jungles, subtropical and tropical countries and areas where tropical diseases are endemic, if not hyperendemic. The civilian population in these regions is being exposed to additional hazards caused by the shifting of military populations, and civilian populations behind areas of military operations are exposed to the potential hazards of disease returning with the moving troops. The mass movement of refugees and of routed armies particularly predisposes to the spread of infectious disease. It is probable that the peak of infection will not reach its maximum until after the cessation of actual warfare, and it is probable that with the ensuing transfer and readjustment of population, a return to the usual prevalence of infectious diseases may not be expected for many years.

With battles raging in all parts of the globe, especially the tropics and subtropics, the military personnel is exposed not only to the classical war diseases but to many new hazards. Most important of these are the infectious tropical diseases<sup>29</sup> which for generations have subjugated native races.

Large numbers of non-immune, susceptible individuals are being concentrated into areas where so-called tropical diseases are prevalent. Although under peacetime conditions,<sup>33</sup> known effective control measures can reduce the rates of many of these diseases to a minimum, under battle conditions it is often impossible to apply preventive measures. The personnel is, therefore, afforded only limited application of control measures and is often exposed to infection of tropical diseases under most trying circumstances. An entire new galaxy of tropical diseases faces the soldier as he struggles through tropical jungles and swamps, through native settlements whose populations are overflowing caldrons of infection.

**Prevalence of Tropical Diseases.** For many years, tropical diseases<sup>47</sup> were regarded as indigenous only in tropic and subtropical areas; but today it is known that the term "tropical"<sup>10</sup> must be interpreted rather as the usual abode of these diseases, but by no means their geographic limitations. Yellow fever, for example, has been epidemic at times in most of the ports along the Atlantic Seaboard as far north as Boston and New Bedford. Fortunately, these outbreaks were limited to the summer and died out with the onset of cold weather.

Filariasis was formerly endemic in South Carolina. Dengue<sup>3</sup> has been endemic along the Gulf of Mexico Coast for generations. Murine typhus<sup>11</sup> has been endemic along the Pacific, Atlantic and Gulf of Mexico Coasts. Cholera has been epidemic in most of America's seaports. Relapsing fever has been reported from the Pacific and western states. Kala-azar (*Leishmania donovani*) has been reported from the southwestern states. Malaria has existed in the United States since the earliest colonial times, and about 1,000,000 new cases are reported annually. In 1935 the death rate from malaria for the United States<sup>43</sup> was 3.5 per 100,000; in 1941 it reached the low rate of only 0.9.

Tropical diseases<sup>29</sup> are more prevalent in the warmer regions, and are due to all types of etiologic agents, bacterium, virus, rickettsia, protozoa or metazoa parasites. Many require particular intermediate hosts and special arthropodal vectors. Some of these diseases are limited by special factors such as climate, distribution of the intermediate hosts, availability of the arthropodal vector<sup>22</sup> or carrier and so forth. A few of these arthropod-borne tropical diseases are a menace to the United States, as malaria.

Other tropical diseases, which are spread by the lack of proper sanitation and personal hygiene, tend to have world-wide distribution in areas of poor sanitation; but these should be of no serious danger to the civilian population of the United States unless there is a breakdown in water and sewage disposal facilities.

Two important problems face civilian physicians and health officers in the United States: (1) the diagnosis and treatment of the returning personnel who may be infected with a tropical disease; (2) the possible importation<sup>27</sup> of tropical disease into the United States.

**DIAGNOSIS AND TREATMENT.** Probably no branch of medicine has advanced as rapidly in diagnosis and treatment as has tropical medicine in the past 50 years. Schools and institutes for research in tropical medicine are increasing in number and in geographic spread, until now almost every country has one or more centers for the study and teaching of tropical medicine.

The Army and Navy medical authorities<sup>28</sup> have felt that the subject of tropical medicine has not been taught with sufficient thoroughness in the medical schools of this country. Consequently, these services have made provisions for supplementary training of their medical personnel in the treatment and control of these diseases. Moreover, a plan for the improvement of the teaching of tropical medicine in the medical schools of the United States and Canada has been developed through the cooperation of the Association of American Medical Colleges, the John and Mary R. Markle Foundation, the Office of the Surgeon-General of the U. S. Army, the National Research Council, the Army Medical School and Tulane University. The plan consists in offering to each medical school an opportunity for 2 members of its staff to attend a course in tropical medicine. Medical schools, in turn, have expanded their teaching of tropical medicine, and many physicians are now treating these diseases, as military activities enter areas of tropical disease prevalence. However, not all cases of tropical diseases will be diagnosed, treated or cured by military physicians. Some patients may not develop symptoms until after discharge and return to their homes, whereas others may have recurrences or unrecognized infections; many of these will come to the family physician for treatment.

Not all diseases<sup>4</sup> contracted in the tropics are necessarily exotic diseases. The attending physician must bear in mind the fact that diseases of the temperate climate also exist in the tropics<sup>46</sup> and that these must be considered in the differential diagnosis of an illness in a patient who has returned from the tropics.

As Strong<sup>47</sup> ably indicates—one of the first questions to be asked a man suspected of having a tropical disease is, "Where have you been during the past months and years?" Thus, from a knowledge of the geographic distribution of diseases, the physician can obtain a clue. The diagnosis of tropical disease, as with other infectious disease, depends upon the patient's history, the physical and laboratory examination, and the physician's knowledge of the disease involved. It has been emphasized that tropical diseases do not necessarily predominate in most tropical countries. Moreover, these diseases may present an unusual or bizarre picture in temperate zones. If the attending physician, after a careful history and examination, suspects the existence of a tropical disease, it is best to call a consultant who is experienced in the diagnosis and treatment of these diseases.

**IMPORTATION INTO UNITED STATES.** The health of the individual is no longer his sole responsibility and affair. The community is concerned

especially with any case of infectious disease which may act as a focus of spread. Certainly, a case of active malaria is of concern to a health officer in a New England community where *Anopheles quadrimaculatus* abound. Similarly, dengue is important to the southern community, and such diseases as bacillary dysentery, cholera, plague or typhus are of concern to every health officer. Recognizing the potential dangers<sup>7</sup> of the importation of tropical diseases, the Army<sup>38</sup> and Navy,<sup>36</sup> in cooperation with the United States Public Health Service, are preparing detailed plans to protect the civilian population against extensive exposure to these diseases. Moreover, these services<sup>25</sup> have taken every known control measure against tropical diseases. Through active research, in cooperation with various civilian groups, they have developed additional protective measures.

*Control by the Army and Navy.* Personnel of the military services<sup>9</sup> receive routine vaccination against smallpox, typhoid and paratyphoid A and B. All military personnel on induction are now immunized, as in the Army,<sup>34</sup> by 3 injections of plain or liquid toxoid or, as in the Navy and Marine Corps,<sup>26</sup> by 2 injections of alum-precipitated tetanus toxoid.

Yellow fever vaccine, manufactured by the United States Public Health Service and the Rockefeller Foundation, is administered to all personnel about to depart for areas in which the disease is prevalent. Under similar circumstances, typhus, cholera and plague vaccines are used. Although the yellow fever vaccine is of proved value, the plague and cholera and typhus vaccines are yet in the experimental stage, but are believed to be of value in the reduction of both mortality and morbidity. General Simmons<sup>42</sup> regards the present immunization program as one of the most potent weapons being used for the preservation of the health of the Army.

The Army<sup>42</sup> established a Preventive Disease Division of the Surgeon-General's office in 1941. The objectives of the division have been the maintenance and conservation of the health of the Army through the prevention and control of infectious diseases and the elimination of sanitary, occupational and other health hazards.

This division includes branches of Sanitation, Sanitary Engineering, Epidemiology, Laboratories, Military Occupational Hygiene, Venereal Disease Control and Medical Intelligence. The Sanitation branch is concerned with the eradication of biting insects, the protection of troops against mites, ticks, lice, bed-bugs, fleas, mosquitoes, sandflies, gnats and biting flies, and with sanitation of water, food and wastes. The Sanitary Engineering branch is concerned with sanitary control of large water purification plants and sewage disposal plants of a permanent nature. With the Corps of Engineers, it initiates and plans insect and rodent control programs for posts in the zone of the interior. In the branch of Epidemiology, the section on tropical diseases initiates programs for the control of these diseases. These programs include the organization of special teams of highly trained control officers, extensive training of medical personnel and the development of new procedures for the control of tropical infections among troops.

A civilian commission on tropical diseases, headed by Dr. Wilbur A. Sawyer, assists and advises the Army in its fight against these diseases. The Medical Intelligence branch collects, assembles and disseminates to troops embarked for a particular area all medical information which may be of value in protecting the troops. For example, if malaria is prevalent in that region, then information as to the anophelines, the breeding and

feeding habits, is included. After collection, the data are studied and used to prepare comprehensive health surveys containing data on topography, climate and population of the country, a section on the public health services and facilities for sanitation and medical care, and detailed information concerning the important diseases and their epidemiology. These surveys are distributed to commanding officers and medical officers of all forces going to foreign countries. They are used as a basis for the specific recommendations made for the protection of all troops sent abroad.

The Navy<sup>23</sup> provides medical officers with special training in tropical medicine, and has recently revised a bulletin "Notes on Tropical and Exotic Diseases of Naval Importance."<sup>24</sup> The purpose of these notes is to present useful and up-to-date information on certain tropical and exotic diseases with which naval medical officers may have had limited experience. The bulletin contains an abundance of maps in color, depicting the geographic distribution of tropical diseases. To Navy personnel 4 groups of disease are of greatest danger: malaria, dengue, dengue-like fevers (sand-fly fevers) and dysenteries (bacillary and amebic). Diseases listed as potential hazards, which do not occur or rarely occur at present among Navy personnel are: typhus and rickettsial diseases, cholera, yellow fever, plague, filariasis, the relapsing fevers, infectious jaundice and other leptospiroses and Bartonellosis (Oroya fever—*Verruga peruana*). Other diseases, such as hookworm infestations, schistosomiasis, trypanosomiasis, leishmaniasis, leprosy, yaws and pinta, are described as of secondary or little importance and are not apt to be of widespread occurrence or to cause great disability among Navy personnel.

Although the possibility of the importation of tropical disease into the United States and in fact to all parts of the world, such as Australia and Great Britain, where these diseases are not usually prevalent, has been overemphasized, nevertheless this hazard does exist. In spite of all the precautions taken by the Army,<sup>19</sup> Navy<sup>20</sup> and the Public Health Service, not all cases will be identified, and not all returning ships and planes will be entirely without danger of transporting insects or rodents which may act as foci.

*Methods of Importation.* A tropical disease may gain entrance into areas where it has not been prevalent by one of several methods:

1. *Disease may be introduced by carriers or cases of which some may not be recognized.* The chances of this type of entrance are greater than ever because of the development of radar and the relative safety of both military and civilian aviation. It is entirely possible for a traveler to leave India and arrive at a Boston airport perfectly well, but later develop bubonic plague which he contracted in India. The problem presented by latent, unrecognized infection and the carrier state among the military personnel cannot be fully appreciated. The danger of importation of disease does not arise from the recognized and treated cases, but from these latent infections and carriers.

2. *Animal reservoirs may be introduced from areas where the disease was indigenous and thereby bring the disease with them.* This is thought to have been the means whereby equine encephalomyelitis<sup>12</sup> was introduced into New England in 1938. Bubonic plague probably entered California by means of infected rats, and now has spread asylvatic plague to many wild rodents in the Pacific and western states as far east as the Mississippi.

3. *Insect vectors may be brought by aeroplanes, ships, or other means of transportation.* Yellow fever was brought into Boston by clipper ship; *Aedes ægypti* mosquitoes bred on shipboard in open casks of fresh water.



The recent outbreak of malaria in Brazil<sup>45</sup> was due to the importation of *Anopheles gambia* from Africa by aeroplane.

Jackson<sup>21</sup> lists cholera, plague, smallpox, typhus, yellow fever, malaria and dengue as diseases of 2 day to 2 week incubation periods, and that they may appear among the passengers of an air transport after their arrival in this country from a port where these diseases are prevalent. Insects, he states, may be carried not only in the interior, but also in depressions of the exterior of the plane. Recently, special disinsectization procedures have been applied to the retractable landing gear of planes, as it was discovered that on retraction within the fuselage an excellent means of hiding was provided for a variety of insects. Recently *Anopheles albimanus* was captured near a Florida airport. As this mosquito is not a native of North America, its means of ingress seems obvious. Several procedures, depending upon the type of disease concerned, are applicable to the control of air-borne transfer of disease: (a) vaccination of personnel and of passengers if indicated; (b) inspection of passengers prior and subsequent to flight; (c) issuance of travel certificates, listing the date and place of departure; (d) spraying of the interior of planes  $\frac{1}{2}$  hour prior to landing and complete internal and external disinsectization upon arrival; (e) inspection of baggage; (f) delousing of clothing and personnel from typhus infested ports.

4. *The disease may find new hosts in the new area.* Examples of diseases which can adapt themselves to a variety of animal hosts are plague, tularemia and brucellosis. Equine encephalomyelitis<sup>12</sup> has been demonstrated in a vast variety of animals, such as horses, mules, domestic fowl, pigeons, ringed neck pheasants and a variety of other wild birds.

5. *The disease may find a new insect vector in the area invaded.* The transmission of yellow fever in different areas is an illustration of the adaptability of various insects to the spread of this disease. Nine species of *Aedes* mosquitoes and some of the members of the genera *Eretmopodites*, *Culex*, *Mansonia* and *Anopheles* have been shown to transmit the virus of yellow fever in West Africa.

6. *New or different strains of the disease may be introduced into an area where less virulent strains were formerly prevalent.* This is illustrated by the introduction of malignant malaria (*Plasmodium falciparum*) into areas where benign malaria (*Plasmodium vivax*) was formerly prevalent. As little or no cross-immunity exists between these 2 types of malaria, the more virulent type will cause a widespread outbreak with many resultant deaths. Similarly, there is the possibility of the importation of the Shiga variety of bacillary dysentery into the United States.

Naturally, here in the United States, health authorities are not so much concerned with the possibilities of tropical disease among the civilian populations of Europe, Asia and Africa as in the possibility of their importation into the States. On this point, one can feel rather reassured; it is extremely unlikely that any tropical diseases, not already prevalent, will become indigenous in this country. In fact, the northern states are relatively safe from most of the tropical infections, and even if latent infections and carriers return to local communities there will be only rare cases, if any, of these exotic infections. The problem in the southern states is, however, more serious.

Some of these so-called tropical diseases are endemic now in this country<sup>34</sup> and, although additional carriers<sup>14</sup> may bring their infections into local communities, few additional cases will result as long as proper sanitation and personal hygiene are maintained. This fact is especially applicable to

the enteric diseases,<sup>2</sup> such as typhoid, paratyphoid, bacillary and amebic dysentery. So long as excreta are properly disposed of, and so long as carriers of these organisms wash their hands thoroughly after going to the toilet and before handling food, there is no danger of an outbreak.

Bacillary dysentery<sup>37</sup> is perhaps one of the most important military diseases of the war. Fortunately, treatment with sulfaguanidine not only shortens the clinical course of the disease, but reduces the length of the carrier stage which usually follows. Nevertheless, asymptomatic carriers<sup>1</sup> and latent infections will occur among returning troops,<sup>40</sup> and there may be an increase in the types of *Shigella* already prevalent; and in addition other types may be introduced. Similarly, *Salmonella* outbreaks may result. Great care must be exercised in preventing<sup>16</sup> outbreaks due to food handlers who are carriers of *Shigella* or *Salmonella* organisms.

Typhus fever, relapsing fever and other louse-borne<sup>15</sup> diseases are another group of tropical infections which are not likely to find a foothold in most of the country. There was a time when some of the immigrants did not regard themselves as virile unless they were lousy—infected with lice. At present, however, body lice are a rare finding in New England, but are still not uncommon among negro and other racial groups, especially in the South and southwestern states. With adequate use of hot water and soap—the American traditional Saturday night bath—the public can remain free of lice and thereby prevent the spread of louse-borne diseases. If the rat population were immensely increased by enemy destruction of cities, and if municipal sanitation standards were broken down, then there might be danger of an outbreak if lice and the infecting organisms were introduced. Under a similar breakdown of sanitation, outbreaks of cholera would be possible. However, since there are good reasons to believe that enemy massive destruction is unlikely, and since the possibility of introducing these infective agents is remote, there need not be concern about such diseases as typhus fever, relapsing fever, bubonic plague and cholera.

Dengue and yellow fever<sup>39</sup> are 2 other diseases which are unlikely to be prevalent, except in the southern states, as the mosquito vector is limited almost entirely to the area south of the Mason and Dixon line, and the infecting organism must be imported. The *Aedes aegypti* mosquito, the vector of these infections, cannot survive northern winters; under most unusual conditions, therefore, it is possible, although highly improbable, that these 2 diseases may gain temporary access even in northern communities. Such outbreaks would naturally disappear spontaneously with the onset of cold weather, and would not reappear again unless reintroduced. Dengue, of course, is endemic in the South, and it is possible that the disease may temporarily become more widespread. However, the United States Public Health Service<sup>35</sup> has undertaken control programs in Texas, Alabama and Florida against *A. aegypti*, and this fact, together with the factors already limiting this disease, would continue to prevent its extension for any great length of time.

Perhaps the disease which may cause most civilian concern in the United States is malaria. Malaria has been endemic throughout most of the United States as far north as Canada. In New England there have been at least 3 epidemic waves, 2 of which spread up the rivers from Long Island Sound to Connecticut and Massachusetts. During the first decade of the 20th century, the disease was still endemic in some parts of New England, but for almost 20 years only rare cases have been reported. In Massachusetts,<sup>17</sup> during the past 14 years, there have been only 11 reported

cases of malaria in persons who were never out of that state and who apparently acquired the disease within the state as a result of mosquito bites. Fortunately, climatic conditions and land improvements have been unfavorable to the continued existence of malaria in New England and the northern states. In the South, intensified malaria-mosquito control programs have been, in the main, responsible for the reduction in malaria infections. With the building of each house and the cultivation of each new farm, additional mosquito-breeding places are destroyed.

At present, health authorities are convinced that the concentration of *Anopheles quadrimaculatus* in the northern states is not sufficient to maintain malaria at an epidemic level. However, if carriers of malaria return, they may act as foci of small local family or neighborhood outbreaks. Adequate treatment of all cases and proper screening of houses are, however, excellent control measures. Both the Army<sup>43</sup> and Navy are treating all cases, and any soldier or sailor who is discharged with malaria is carefully followed up by the state departments of public health. All measures are taken to protect the public.

In the South<sup>44</sup> the danger from malaria is, of course, much more serious, as the concentration of *A. quadrimaculatus* is far greater, the mosquito season is longer, and screening of houses is not as adequate. The United States Public Health Service has taken cognizance of this fact and through its program, "Malaria Control in War Areas,"<sup>45</sup> has limited all of its mosquito-control work south of the Mason and Dixon line. North from here, the "M. C. W. A." has limited its work to mosquito surveys in the immediate environs of military hospitals and institutions where patients ill with malaria are likely to be hospitalized.

A few cases of plague, cholera, leprosy, yaws, leishmaniasis, trypanosomiasis, schistosomiasis, or other helminth infections may occur among troops coming into close contact with native populations. Phlebotomus fever and filariasis may incapacitate a considerable number for future military service in the tropics, but it is not likely that the latter will become endemic again in this country. These diseases will present problems of diagnosis and treatment, but are not likely to gain endemicity or even temporary epidemicity in the United States. As Meleney<sup>32</sup> well summarizes: "It may be stated with considerable confidence that the chief hazards in the importation of exotic diseases are from the introduction of new strains of parasites already present, and the introduction of new vectors of disease."

**Conclusion.** Wars have always been accompanied by increases of infectious diseases. In this global war, zones of military operations are extended into areas where tropical diseases are prevalent. American forces face not only the hazards of battle but the classical war diseases augmented by limitation in the application of control measures against the more exotic diseases. Although the Army, Navy and United States Public Health Service are taking all steps to protect our military personnel and prevent extension of tropical diseases to the civilian population, not all cases will be detected before discharge.

The civilian physician is, therefore, faced with the diagnosis and treatment of discharged service men who may have contracted tropical disease. Simultaneously, health authorities are concerned with the possible importation of these diseases. Fortunately, tropical diseases are not likely to bring about any major catastrophe in this country. Because of climatic and environmental factors, tropical diseases do not find favorable conditions for epidemic prevalence. The same factors, which in the past have been gradually bringing about the diminished endemicity of malaria and dengue, will continue to operate and will be aided by extension of control

measures if these are indicated. Malaria is indubitably the most serious problem, and even this disease is not likely to cause extensive outbreaks so long as health authorities are prepared to instigate intensive control measures. Perhaps the only diseases, which will cause any significant increase in incidence, are the enteric infections such as salmonella and shigella. The other tropical diseases are not a menace to the public and, although sporadic cases and even localized outbreaks may occur, not too much concern need be expressed. It is well to remember that military and health authorities are cognizant of the dangers and that all possible steps are being taken to protect the civilian population against the importation of tropical diseases.

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## PHYSIOLOGY

## PROCEEDINGS OF

## THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF JANUARY 18, 1944

"Bound" Sulfonamide Compounds in Plasma With Special Reference to Renal Clearance of Various Sulfonamides. J. G. REINHOLD, H. F. FLIPPIN, A. H. DOMM and L. POLLACK (Biochemical Laboratory, Phila-

delphia General Hospital). A variable but often considerable fraction of sulfonamide compounds present in plasma fails to filter under pressure through cellophane membranes. The most important factor determining the proportion so retained, besides the structure of the sulfonamide compound itself, is the concentration of protein in plasma, especially the albumin. Sulfonamide so bound in a protein-sulfonamide complex appears to be in equilibrium with the free sulfonamide and plasma protein. The penetration of sulfonamides into erythrocytes and other cells, and into cerebrospinal and other body fluids, including the glomerular filtrate, is confined to the ultrafiltrable fraction. The renal clearance of sulfathiazole in man, when calculations are based upon its concentration in plasma ultrafiltrate, is equal to the inulin clearance. Thus sulfathiazole is excreted solely by the glomeruli without tubular secretion or reabsorption. Sulfadiazine and sulfapyridine, on the other hand, are reabsorbed to a considerable extent by the tubules.

The ultrafiltrability of serum sulfamerazine has been studied in a group of patients receiving treatment for pneumonia or meningococcic meningitis. Strikingly decreased ability to bind sulfamerazine has been observed in some individuals in the presence of exceptionally severe or fatal illness.

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**Tests of Pyrogenicity, Antigenicity, and the Efficacy of Ossein Gelatin Preparations\* in Repeated Massive Hemorrhage and Infusion.** W. M. PARKINS, L. H. SAXE and H. M. VARS (Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania). Standard pyrogenicity tests of 8 different lots of 4 and 6% gelatin solutions prepared specifically for infusion purposes were negative.

Ossein gelatin (Lot P 4-20) has been tested serologically in rabbits and for anaphylactic symptoms in rabbits and guinea pigs after sensitizing doses of gelatin and beef serum. This gelatin apparently is non-antigenic and free from sensitizing impurities.

Forty-seven dogs, under light nembutal anesthesia, were bled (4 cc. per kilogram per minute) to a blood pressure end-point of 20 mm. Hg and then immediately infused with a volume of saline, gelatin or pooled heparinized dog plasma equal to the bleeding volume. One hour after initial hemorrhage in some groups, and 3 hours after infusion in others, a 2nd hemorrhage was performed, at the same rate and to the same end-point as the initial hemorrhage.

The average bleeding volume (cc. per kilogram) of the 2nd hemorrhage expressed as percentage of initial bleeding volume (5 dogs) was as follows: (a) 1 hour after initial hemorrhage—saline, 49; 4% gelatin (P 4-20), 100, (P 1-20), 95 and (P 2-20), 100; plasma, 75; 6% gelatin (P 7-20), autoclaved 20 minutes, 117, and (P 7-180), autoclaved 180 minutes, 108. (b) 3 hours after 1st infusion—gelatin (P 7-20), 84; (P 7-180), 65; saline, 36; plasma, 65.

The more marked hemodilution (hematocrit) and increase in blood pressure, as well as a higher concentration of plasma gelatin after infusion of 6% gelatin autoclaved 20 minutes, as compared with the 4% and 6% autoclaved 180 minutes, support the following conclusions from bleeding volume determinations: (1) 6% solutions appear more effective than 4% gelatin. (2) Prolonged autoclaving which markedly decreases colloid size, gelatin and viscosity, and increases osmotic pressure, somewhat

\* Gelatin preparations were supplied by Dr. D. Tourtellotte of the Chas. B. Knox Gelatine Co., Johnstown, N. Y.

reduces the efficacy, as determined at the 1 and 3 hour intervals. (3) All these gelatin solutions compared favorably with plasma in these hemorrhage experiments where infusion is immediate.

According to the criteria used in experiments now in progress, plasma or whole blood appears to be superior to gelatin when a period of severe hypotension (30 to 35 mm. Hg) is extended to 1 hour before infusion, at which time factors in addition to blood volume, such as peripheral resistance, myocardial and metabolic deficiencies may be involved.

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**Some Clinical Experience With Gelatin as a Plasma Substitute.** C. E. KOOP, A. G. FLETCHER, JR., and C. RIEGEL (Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania). One hundred patients have been given 190 intravenous infusions of an especially prepared ossein gelatin totaling 132 liters. The toxicity studies on dogs previously reported to this society have been extended to human subjects. Observations have been made on the effect of single and repeated intravenous gelatin infusions on serum proteins, bromsulphalein retention, urea clearance, blood coagulation and sedimentation rate.

There is a reciprocal relation between the concentration of serum proteins and gelatin protein in the circulating blood after gelatin infusion. The serum protein values are temporarily depressed and rapidly return to normal as the gelatin disappears from the blood stream over a period of 3 to 5 days. Bromsulphalein retention did not occur, and urea clearance tests indicated no renal impairment after infusions of 1000 cc. in 90 minutes. Blood coagulability was not altered, but the sedimentation rate was increased. Histologic examination of autopsy material revealed minor changes similar to those previously reported in dogs.

Clinical studies included observations on blood pressure, pulse, respiration and temperature. No pyrogenic or antigenic reactions were seen. Venous pressure was consistently elevated and cardiac output was increased. Gelatin was used therapeutically in the treatment of acute hemorrhagic shock and edematous states and as a general postoperative measure in surgical cases.

The following conclusions were reached: Intravenous gelatin solutions are easily and safely administered to human subjects. The material is an effective agent in the treatment of at least the early stages of hemorrhagic shock. After major operative procedures the administration of gelatin produces a favorable response similar to that observed with plasma infusions. In certain types of edema there is, following the administration of gelatin, a diuresis which is accompanied by clinical improvement. Further studies on the value of gelatin in shock, burns and hypoproteinemic states are clearly warranted.

# BOOK REVIEWS AND NOTICES

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**THE FOOT.** By NORMAN C. LAKE, M.D., M.S., D.Sc. (LOND.), F.R.C.S. (ENG.), Senior Surgeon and Lecturer on Surgery, Charing Cross Hospital; Surgeon, Bolingbroke Hospital; Consulting Surgeon, Emergency Medical Service; Director of Studies, London Foot Hospital; etc. Third Ed. Pp. 432; 136 figs. Baltimore: The Williams & Wilkins Company, 1943. Price, \$5.00.

THIS little treatise on the foot is excellent. The author spends much time on the evolution of the foot and reviews the disabilities whether they are due to congenital causes, infections or injuries. He achieves a well-balanced book which can be profitably used by the medical student and general practitioner. He handles the matter of the various operations at times necessary on the foot with well-balanced judgment. This third edition provides an opportunity for the author to include his comments upon foot disabilities from wartime causes.

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**SYMPTOMS AND SIGNS IN CLINICAL MEDICINE.** An Introduction to Medical Diagnosis. By E. NOBLE CHAMBERLAIN, M.D., M.Sc., F.R.C.P., Lecturer in Medicine, University of Liverpool; Physician to Out-patients, Royal Liverpool United Hospital, Royal Infirmary Branch; Visiting Physician, Smithdown Road Hospital, Liverpool. Third Ed. Pp. 456; 346 illus. (19 colored). Baltimore: Williams & Wilkins Company, 1943. Price, \$8.00.

THE appearance of the war edition of this book is made possible only by the English publisher's "overcoming insuperable difficulties after repeated destruction of their works by bombing." Nazi bombs failed either to stop the printing or the efforts of the authors in revising this book. In the 5 years elapsed since the last edition, considerable revision has been required. Among the main changes are the additions to the section on endocrine defects, amplification of the symptoms and signs of respiratory disease and extensive revision of the chapter on the cardiovascular system. Of war interest is Dr. A. R. D. Adams' useful details on tropical parasites.

The author, however, has retained the rightful importance given to physical signs observed by the unaided eye in the diagnosis of disease. Dr. Chamberlain's attitude in writing each chapter before consulting standard textbooks and monographs is highly commendable, for it represents what a physician can carry in his mind. The book, therefore, is valuable to first-year clinical students in giving them the information essential in making a physical diagnosis.

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**PATHOLOGICAL HISTOLOGY.** By ROBERTSON F. OGILVIE, M.D., F.R.C.P. (EDIN.), Lecturer in Pathology and Assistant in Forensic Medicine, University of Edinburgh; Senior Pathologist, Royal Infirmary, Edinburgh; Pathologist to the Leith and Deaconess Hospitals, Edinburgh; Examiner in Pathology and Forensic Medicine for the Triple Qualification. Second Ed. Pp. 411; 235 photomicrographs in color. Baltimore: Williams & Wilkins Company, 1943. Price, \$9.00.

THIS book retains all of the excellent features of the first edition, has many new illustrations, and new matter on pathologic changes in intestinal tract, kidneys, bones, and reproductive organs, and much has been rewritten and amplified. In spite of these additions, the book is slightly smaller and lighter in weight than the first edition.

All of the 235 photomicrographs are in color, are of superb technique, and are well chosen as representative examples of common pathologic conditions. This book is intended as a companion to a standard textbook of pathology, to aid students in studying their microscopic slides. Although the emphasis is on the clear, simple description of histologic changes, there is always a preceding brief description of gross pathology, etiology and pathogenesis. Uncommon conditions are not included.

The only criticism the Reviewer would make would be in the matter of terminology; for instance, it seems unwise to use such terms as "glycogen degeneration" and "tubular nephritis." But these are minor matters and do not detract from the usefulness of this volume in helping the student to see the common microscopic changes of disease.

I. Z.

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PHYSIOLOGICAL PSYCHOLOGY. By CLIFFORD T. MORGAN, Associate in Psychology, The Johns Hopkins University; Formerly Faculty Instructor in Psychology, Harvard University. Pp. 623; 176 figs. New York and London: McGraw-Hill Book Company, Inc., 1943. Price, \$4.00.

GREAT advances in a field of science are made when the methods of experimentation become more simplified, more easily reproducible, and more quantitative. As the experimental facts accumulate, however, and new concepts are developed, further advances often require correlation of ideas with other fields of investigation.

This book has been written as a presentation of the known experimental facts important in the understanding of certain concepts of the physiologic mechanism of normal human and animal behavior. Of these, 3 have been stressed by the author—*internal environment*, *interaction*, and *levels of organization of function*. This has been done so well that workers in other fields, especially biochemistry, can with little difficulty determine whether certain psychologic problems fall within the realm of their experimental technics and understanding. "This book is meant to be both a textbook for under-graduates who are preparing for psychology or medicine and a reference book for graduate students and workers in psychology, physiology, and medicine." In the Reviewer's opinion, however, the revolution expected in psychology as a result of the impact of biochemistry, so often heard in psychological circles, can certainly spring from this book as its manifesto.

The difficulties of writing a textbook of this type lie chiefly in the choice between an elementary presentation of factual material and a scientific presentation from the investigator's point of view who demands an interpretation of the facts in light of the limitation of experimental procedures. The author has overcome these difficulties by presenting the elementary material of psychology, physiology and anatomy in the first part of the book, and then discussing the more advanced research, with complete bibliographic references (780), in the rest of the book. The bibliography also contains an author index whereby the page upon which that reference is made is found with the bibliographic reference. This is an extremely useful index procedure.

J. S.

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PERSONAL AND COMMUNITY HEALTH. By C. E. TURNER, A.M., Sc.D., DR.-P.H., Professor of Public Health in the Massachusetts Institute of Technology; Formerly Associate Professor of Hygiene in the Tufts College Medical and Dental Schools; Sometime Member of the Administrative Board in the School of Public Health of Harvard University and the Massachusetts Institute of Technology. Seventh ed. Pp. 585; many figs., 4 colored plates. St. Louis: C. V. Mosby Company, 1943. Price, \$3.50.

THE author presents another edition of an outstanding text for health instruction of college and university levels. It incorporates recent advances in the fields of public health and medical sciences with the basic fundamentals



of each, without burdening the student with unnecessary details. The book has been developed over a period of many years of personal experience in health instruction to students of various age levels and from suggestions offered by co-workers and colleagues in the field of public health and hygiene. A book of this type could certainly be utilized advantageously in college courses in personal and community health and as a text in an introductory course in the biologic sciences.

F. E.

## NEW BOOKS

*The Medical Clinics of North America.* Chicago Number. Symposium on Cardiovascular Diseases. Pp. 289; 37 figs.; 7 tables. Philadelphia: W. B. Saunders Company. Price, \$16.00 per year.

*The Sexual Glands of the Male.* By OSWALD SWINNEY LOWSLEY, A.B., M.D., F.A.C.S., Collaborators: FRANK HINMAN, A.B., M.D., F.A.C.S., DONALD R. SMITH, A.B., M.D., and ROBERT GUTIERREZ, A.B., M.D., F.A.C.S. Reprinted from Oxford Loose-Leaf Urology. Pp. 619; 4 tables; 59 figs. New York: Oxford University Press. Price, \$10.00.

*On the Influence of Trades, Professions, and Occupations in the United States, in the Production of Disease.* (Reprinted from Transactions of the Medical Society of the State of New York, Vol. III, 1837, pp. 91-150.) By BENJAMIN W. MCCREADY, M.D. Introductory Essay by GENEVIEVE MILLER, M.A. (Publications of the Institute of the History of Medicine, The Johns Hopkins University. Fourth Series: Bibliotheca Medica Americana, Vol. 4.) Pp. 127. Baltimore: The Johns Hopkins Press. Price, \$1.75.

*Pathology and Therapy of Rheumatic Fever.* By LEOPOLD LICHTWITZ, M.D., Lately, Chief of the Medical Division of the Montefiore Hospital, and Clinical Professor of Medicine, Columbia University, New York City. Foreword by WILLIAM J. MALONEY, M.D., LL.D., F.R.S. (EDIN.), Consulting Neurologist to the City Hospital; Formerly Professor of Nervous and Mental Diseases, Fordham University, New York City. Edited by MAJOR WILLIAM CHESTER, M.C. Pp. 211; 69 figs. New York: Grune & Stratton, Inc., 1944. Price, \$4.75.

*The Health of Children in Occupied Europe.* Published by the International Labour Office. Pp. 37; 2 tables. Montreal, Canada, 1943. Price, 25c (one shilling).

A small pamphlet studying "The effects of the war on the health of the children (in occupied Europe). . . . Because of the difficulty of collecting precise and reliable information . . . the data quoted are fragmentary and serve only to illustrate the state of affairs."—Introduction.

*Office Treatment of the Nose, Throat and Ear.* By A. R. HOLLENDER, M.Sc., M.D., F.A.C.S., Associate Professor of Laryngology, Rhinology and Otolaryngology, University of Illinois College of Medicine; Otolaryngologist, Research and Educational Hospitals, Chicago, Ill. Pp. 480; 33 figs. Chicago: The Year Book Publishers, Inc., 1943. Price, \$5.00.

*Physical Biochemistry.* By HENRY B. BULL, Ph.D., Associate Professor of Physiological Chemistry, Medical School of Northwestern University. Pp. 347; 93 figs.; 44 tables. London: Chapman & Hall, Ltd.; New York: John Wiley & Sons, Inc., 1943. Price, \$3.75.

*The Permeability of Natural Membranes.* By HUGH DAVSON, D.Sc., Associate Professor of Physiology at Dalhousie University, Canada; formerly Demonstrator in Biophysics and Beit Memorial Fellow, University College, London; and JAMES FREDERIC DANIELLI, D.Sc., A.I.C., Beit Memorial Research Fellow and Fellow of St. John's College, Cambridge, England. Foreword by E. NEWTON HARVEY, Professor of Physiology in Princeton University. Pp. 361; 73 figs.; 72 tables. Cambridge: At the University Press; New York: The Macmillan Company, 1943. Price, \$4.75.

- A Catalogue of the Medieval and Renaissance Manuscripts and Incunabula in the Boston Medical Library.* Compiled by JAMES F. BALLARD, Director, Boston Medical Library. Pp. 246; 23 figs. Boston: Privately printed.
- Human Constitution in Clinical Medicine.* By GEORGE DRAPER, M.D., Associate Professor of Clinical Medicine, College of Physicians and Surgeons, Columbia University, and Associate Attending Physician, Presbyterian Hospital, New York City; C. W. DUPERTUIS, Ph.D., Physical Anthropologist, Constitution Clinic, Presbyterian Hospital, New York City; and J. L. CAUGHEY, JR., M.D., MED.SCI.D., Associate in Medicine, College of Physicians and Surgeons, Columbia University; Assistant Physician, Presbyterian Hospital, New York City. Pp. 273; 29 figs.; 30 tables. New York and London: Paul B. Hoeber, Inc. Price, \$4.00.
- Health and Hygiene.* A Comprehensive Study of Disease Prevention and Health Promotion. By LLOYD ACKERMAN, Western Reserve University. Pp. 895; 59 figs.; 27 tables. Lancaster, Pa.: The Jaques Cattell Press, 1943. Price, \$5.00.
- The British Encyclopædia of Medical Practice* including Medicine, Surgery, Obstetrics, Gynecology, and Other Special Subjects. *Medical Progress*, 1943 (Pp. 382); *Cumulative Supplement*, 1943 (Pp. 342; 5 figs.). Under the General Editorship of SIR HUMPHRY ROLLESTON, BT., G.C.V.O., K.C.B., M.D., D.Sc., D.C.L., LL.D., Emeritus Regius Professor of Physic, Cambridge; Sometime President of the Royal College of Physicians of London. London, Eng., and Toronto, Can.: Butterworth & Co. (Publishers), Ltd., 1943.
- Soviet Health Care in Peace and War.* By ROSE MAURER. The American Russian Institute for Cultural Relations With the Soviet Union, Inc. Pp. 48; a few illus. New York: American Russian Institute. Price, 10c.
- An informative pamphlet on the remarkable medical achievements of Soviet Russia in recent years, written to illustrate the application of the principle that health is a community affair.—E. K.

## NEW EDITIONS

- An Outline of General Physiology.* By L. V. HEILBRUNN, Professor of Zoölogy in the University of Pennsylvania. Second ed. Pp. 478; 135 illus. Philadelphia and London: W. B. Saunders Company, 1943. Price, \$6.00.
- Urology for Nurses.* By OSWALD SWINNEY LOWSLEY, M.D., F.A.C.S., Director of the Department of Urology (James Buchanan Brady Foundation) of the New York Hospital; and THOMAS JOSEPH KIRWIN, M.D., F.A.C.S., Attending Urologist of the Department of Urology (James Buchanan Brady Foundation) of the New York Hospital. New Printing. Pp. 493; 108 illus. Philadelphia: J. B. Lippincott Company, 1943. Price, \$3.00.
- Chemistry of Organic Medicinal Products.* By GLEN L. JENKINS, Dean and Professor of Pharmaceutical Chemistry, School of Pharmacy, Purdue University; and WALTER H. HARTUNG, Professor of Pharmaceutical Chemistry, School of Pharmacy, The University of Maryland. Second ed. Pp. 675; 71 tables; many small figs. New York: John Wiley & Sons, Inc., 1943. Price, \$6.50.
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# THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

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## ORIGINAL ARTICLES

### "CARDIAC" OR CONGESTIVE CIRRHOSIS PATHOLOGIC AND CLINICAL ASPECTS

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The purpose of this study was to determine the nature of the fibrosis of the liver in chronic passive hyperemia, its frequency, and its relation to various forms of cardiac disease. A diagnosis of cirrhosis was accepted only when, in addition to fibrosis, there was definite alteration of architectural pattern. In certain instances, the fibrosis and structural alterations were comparatively slight rather than well marked, and focal rather than widespread. Any borderline cases of this sort were not included as cirrhotic livers. The term cardiac cirrhosis has gained widespread usage. However, congestive cirrhosis is a more accurate designation since it applies directly to the condition in the liver.

**Materials and Methods.** The cardiac diseases included endocarditis lenta, syphilitic heart disease, cor pulmonale, coronary thrombosis, hypertensive heart disease, chronic congestive pericarditis, and rheumatic heart disease, divided as to forms into cases of aortic stenosis, of mitral stenosis, and combined valvular lesions. Mitral stenosis was always present in the cases of combined valvular disease together with deformity of either or both the aortic and tricuspid valves. The syphilitic heart disease included aortic insufficiency. The cases of coronary thrombosis showed remote or recent occlusion with myocardial infarction, and in 12 of these cases there was associated hypertension.

A group of 25 cases was selected for each type of cardiac disease except congestive pericarditis, in which only 8 were available. This gave a total of 208 cases. In every instance the heart disease was the primary clinical lesion and the patient showed congestive failure. After the number of cirrhotic livers in each type was determined, a survey was made of the 4200 consecutive autopsies from which the various groups were drawn, and the additional cases of cirrhosis included in the study. Microscopically, sections from both lobes of the liver, stained with hematoxylin and eosin, were examined. Stains for iron-containing pigment, and

for connective tissue by the azan carmine method were made in all cases. In some instances stains were prepared for reticulum.

The livers were studied for thickening and condensation of stroma, degree and distribution of fibrosis, necrosis and regeneration of parenchyma, and distortion of lobular pattern.

Weights of the liver and spleen were recorded, also the presence at autopsy of ascites, jaundice, edema, and obstruction of the portal circulation.

The clinical data for all cases included duration of cardiac failure, number of attacks of decompensation, palpability of the liver and spleen, ascites, jaundice (icterus index level) and blood proteins.

**Results.** There were 21 livers with cirrhosis among the 208 cases of cardiac disease. These were distributed as follows: 1 each in the groups of cor pulmonale, coronary thrombosis (this patient also had hypertension), and rheumatic heart disease with aortic stenosis; 2 in the group of hypertensive heart disease; 5 in patients with chronic constrictive pericarditis; 4 in cases of rheumatic heart disease with pure mitral stenosis, and 7 in rheumatic cases with combined valve lesions.

Nine additional instances of congestive cirrhosis were found in reviewing the 420 autopsies covering the study. There were 3 cases in patients with rheumatic heart disease, 2 in patients with hypertension, and 1 each in a case of arteriosclerotic heart disease, combined hypertension and cor pulmonale, and combined rheumatic and congenital heart failure of unknown etiology.

The total of 30 cases of congestive cirrhosis was distributed as shown in Table 1.

TABLE 1.—DISTRIBUTION OF 30 CASES OF CONGESTIVE CIRRHOSIS AMONG VARIOUS TYPES OF HEART DISEASE IN 420 AUTOPSIES

Type of heart disease	Number of cases of congestive cirrhosis
Cor pulmonale	1
Coronary thrombosis	1
Hypertensive heart disease	4
Chronic constrictive pericarditis	5
Rheumatic heart disease:	
Aortic stenosis	1
Mitral stenosis	5
Combined valvular disease	9
Arteriosclerotic heart disease	1
Hypertension and cor pulmonale	1
Rheumatic and congenital heart disease	1
Heart disease of unknown etiology	1
Total	30

The groups in which there was the slightest degree of hepatic fibrosis were endocarditis lenta, coronary arterial disease, and aortic stenosis. The livers generally revealed thickening or condensation of the intra-lobular stroma. Fibrosis was observed in only a few instances and consisted of small foci in the portal or central zones of the lobules. Fibrosis of the liver was more prominent in the cases of syphilitic heart disease, cor pulmonale, and hypertensive heart disease, especially the latter. Many livers of the syphilitic group showed condensation

of stroma, accompanied in 5 instances by focal proliferation of fibrous tissue. In cases of cor pulmonale and hypertension, focal hepatic fibrosis of slight or moderate degree was frequent and there was a tendency to anastomosis of fibrous tissue between lobules. The latter was sufficiently widespread to diagnose cirrhosis in 1 case of cor pulmonale and in 2 of hypertension. Moreover, 3 hypertensive cases showed a degree of fibrosis consistent with a diagnosis of slight cirrhosis.

The livers of the patients with rheumatic heart disease were frequently the seat of fibrosis. The increase in connective tissue varied from slight to marked and was focal or moderately diffuse. Four of the 25 patients with pure mitral stenosis and 7 of the 25 with combined valvular disease had congestive cirrhosis. Of the total of 30 cirrhotic livers, 15 occurred in patients with rheumatic heart disease.

The most severe grade of hepatic fibrosis occurred in patients with chronic constrictive pericarditis. Five of the 8 livers from patients with this cardiac lesion were the seat of definite cirrhosis and in 2 others there was slight cirrhosis.

Of the 30 cases 16 were in males and 14 in females. There was 1 colored patient and the rest were white. The range of age distribution was wide (14 to 74 years), but the majority of patients were between 30 and 50 years. All patients with cirrhosis had congestive heart failure, generally severe and of long duration, i. e., 1 to 14 years. In 10 cases there was continuous cardiac insufficiency, apparently without significant improvement, for a period of 1 to 10 years. In 20 cases there was a history of repeated episodes of decompensation. This was especially true of the rheumatic group in which the number of hospital admissions for decompensation ranged from 3 to 14, usually over a period of years.

TABLE 2.—COMPARISON OF CASES OF CHRONIC PASSIVE HYPERTENIA OF THE LIVER WITH CASES OF CONGESTIVE CIRRHOSIS

Type of heart disease	Weights of			Weights of			Weights of			Weights of		
	Liver			Liver			Liver			Liver		
	No. of cases	1501 gm. or less	2000 gm. or less	No. of cases	1501 gm. or less	2000 gm. or less	No. of cases	1501 gm. or less	2000 gm. or less	No. of cases	1501 gm. or less	2000 gm. or less
Endocarditis lenta.	25	5	12	8	2	23	2	11	11	8	2	2
Syphilitic heart disease.	25	11	12	2	14	11	11	11	11	11	11	11
Cor pulmonale.	25	16	7	1	18	6	6	11	11	11	11	11
Coronary thrombosis.	24	14	9	1	20	4	4	10	10	10	10	10
Hypertensive heart disease.	23	8	11	4	18	5	5	10	10	10	10	10
Chronic constrictive pericarditis.	3	2	1	0	18	1	1	10	10	10	10	10
Rheumatic heart disease:												
Mitral stenosis.	21	12	10	2	14	10	10	10	10	10	10	10
Aortic stenosis.	24	13	7	1	18	6	6	11	11	11	11	11
Combined valvular disease.	18	10	8	0	10	9	9	11	11	11	11	11
Total.	187	91	77	19	107	80	80	17	17	17	17	17
* At least 250 cc. of fluid were required for this diagnosis.												

Table 2 gives the weights of the liver and spleen, also the presence of ascites and jaundice in the 30 cases of cirrhosis. Similar data are recorded for 187 livers, the seat of chronic passive hypertension. The latter represent the non-cirrhotic livers in the various etiologic types

of heart disease. The figures show that, in the cirrhotic group, there is a tendency to smaller livers, larger spleens, and more frequent incidence of jaundice. A few large spleens, weighing up to 650 gm., were present in both groups.

**Comment.** The 5 cirrhotic livers from patients with chronic constrictive pericarditis showed the most extensive fibrosis and distortion of architectural pattern of the entire group. Increase of connective tissue was especially prominent in the portal zones, together with proliferation of bile ducts. All the patients had prolonged hepatic venous stasis due to mechanical obstruction to venous outflow. Cardiac incompetence was more or less continuous for a period of 2 to 10 years. In the 3 cases without cirrhosis, cardiac failure was of shorter duration (4 to 14 months). Six patients died shortly following pericardiectomy and 1 patient died of unrelated cause 21 months after operation. Compared with other forms of heart disease, chronic constrictive pericarditis is infrequent.

Congestive cirrhosis is relatively common in rheumatic heart disease. It is found only in connection with marked valvular deformity. Except for 1 case of pure aortic stenosis, all patients in this group had "button-hole" mitral valves. The latter occurred either alone or in combination with deformity of other valves, especially aortic and tricuspid. In 3 cases there was stenosis and insufficiency of all four valves. The patients in this group with cirrhosis had in common a long history of cardiac incompetence with periods of improvement and failure.

Our survey of 4200 autopsies showed 103 cases of marked rheumatic valvular deformity, in 15 of which congestive cirrhosis was present. Omitting 25 instances of pure aortic stenosis, the 15 cirrhotic livers occurred among 78 rheumatic hearts, an incidence of 19%. Breaking the figures down further showed 5 cirrhotic lesions among 32 patients with pure mitral stenosis (16%) and 8 lesions among 46 patients with combined valvular disease (17%).

There were 30 patients with tricuspid stenosis, 7 of whom (23%) had congestive cirrhosis. Although the incidence of cirrhosis is somewhat higher in cases with tricuspid stenosis, the latter is probably not of significance *per se*, since narrowing of the valve lumen is generally slight. The important consideration is that patients with tricuspid stenosis have had recurrent attacks of rheumatic fever and developed marked deforming rheumatic heart disease, usually polyvalvular, with cardiac failure of long duration.

On a percentage basis, cirrhosis occurs less frequently in hypertensive than in rheumatic heart disease. However, the livers of hypertensive patients often show a definite tendency to fibrosis. The latter is usually slight or moderate, focal rather than diffuse, and prominent in portal location. The last may result in an incorrect diagnosis of early Laennec's cirrhosis.

The cirrhotic lesion is uncommon in cor pulmonale in spite of primary right ventricular failure. Patients with this lesion show progressive dyspnea, often for several years, but frank decompensation is



usually terminal. Also, the pulmonary disease probably accounts in part for the long history of dyspnea.

Congestive cirrhosis is uncommon or rare in patients with pure aortic stenosis, syphilitic aortic regurgitation, coronary thrombosis, or endocarditis lenta. In aortic stenosis or regurgitation, cardiac failure is often abrupt and its duration comparatively brief with few or no intervals of improvement. In some cases of coronary thrombosis in this study, heart failure followed an acute thrombotic occlusion and was of short duration. Other patients had survived episodes of occlusion up to 8 years previously and showed one or more healed infarcts at autopsy. Repeated attacks of decompensation were uncommon. Patients with endocarditis lenta, in whom long-standing heart failure is rare, showed the least severe grade of passive hyperemia of the liver. Death was generally due to the effects of bacteremia and embolism.

**Incidence of Congestive Cirrhosis.** Among 4200 consecutive autopsies at this institute, there were 164 cases of cirrhosis of the liver (4%). Of the four main types, there were 74 cases of Laennec's cirrhosis, 31 of obstructive biliary type, 30 of congestive cirrhosis, and 15 of toxic cirrhosis. The congestive lesion comprised 18% of the cirrhotic livers and occurred in 0.7% of the total number of autopsies.

These figures are similar to those of Ophüls,<sup>12</sup> who gave an incidence of 0.7% for congestive cirrhosis, *i. e.*, 22 cases among 3000 autopsies, and to those of Boles and Clark<sup>2</sup> who had 33 cases among 4000 autopsies (0.8%). The latter found congestive cirrhosis second in frequency to the Laennec type. In 6548 autopsies reviewed by Garvin,<sup>5</sup> there were 790 adults dead of heart disease and 35 (4.4%) of these had congestive cirrhosis. Out of 75 cases of heart disease with either a prolonged single or multiple episodes of congestive failure, Boland and Willis<sup>1</sup> accepted a diagnosis of cirrhosis in 5 (7%). Menne and Johnston<sup>11</sup> reported 17 cases of congestive cirrhosis among 6500 autopsies but did not include them in the category of true cirrhosis.

**Morphology.** Grossly, the liver of congestive cirrhosis is firm, retains the usual shape, and is normal or slightly reduced in size. The capsule is thickened, opaque, and slightly uneven or nodular. The color varies from the usual purplish brown to brownish or yellowish red. In our 30 cases the weights ranged from 850 to 2600 gm.; 20 livers weighed 1500 gm. or less, while 7 weighed from 1501 to 2000 gm.

The organ cuts with increased resistance, revealing a nodular cross-section which shows diffuse alteration of the lobular structure. Discrete or confluent gray or yellowish gray nodules from 1 to 5 mm. in diameter, elevated and irregular in outline, are separated by interlobular brownish red retracted zones of varying size and shape. When confluent, the nodules form prominent foci isolated among the hyperemic zones, and appear to be composed of enlarged and distorted liver lobules. Usually no bile staining is present, but in some instances patchy yellow or yellowish green foci are observed, especially in the firm retracted zones. The appearance sometimes resembles that of the so-called nutmeg liver, which may represent a stage in the development of cirrhosis.

Microscopically, the central zones show slight to marked hyperemia. Sometimes there is accumulation of blood which obliterates the architectural pattern. The changes in the liver cells range from slight atrophy to degeneration or necrosis. The necrotic zones vary in size and shape, and often show an asymmetric distribution around the central veins. They may be situated primarily to one side of these veins, and extend to the periphery of the lobule. Anastomosis of hypereemic foci in adjacent lobules produces an irregular pattern in which portal spaces are isolated or joined to central zones.

The stroma of the central zones is thickened and prominent. There is an increase of reticulin and deposit of intercellular collagen to form compact fibrous bands. Fibrosis also occurs in the portal regions, accompanied by proliferation of bile ducts and infiltration of lymphocytes. These changes are sometimes pronounced. Both the portal zones and the intralobular stroma serve as a source of connective tissue. Although generally observed in both central and portal regions, the fibrosis may predominate in either one. The connective tissue is usually compact and relatively acellular, but active growth is indicated by the presence of young fibroblasts in both regions. In well-developed cases, bands of fibrous tissue extend within the lobules from the central to the peripheral zones and also connect central or portal zones in adjacent lobules.

The so-called regenerative foci, which correspond to the elevated gray or yellowish gray nodules in the gross, are composed of parts of adjacent lobules or whole lobules, especially the peripheral zones. There are groups of large liver cells, irregular in outline and containing abundant granular cytoplasm. The cord arrangement of the cells is usually preserved. Only slight hyperemia and fibrosis occur in such foci. Cellular activity is indicated by the presence of single or double nuclei ranging from small and hyperchromatic to large and vesicular. However, since actual cell division is generally not demonstrable, the degree of parenchymal regeneration is uncertain. The changes may be largely functional and due to hypertrophy of less injured cells.

The usual lobular pattern is altered. Portal spaces undergo eccentric change in position and several of them are often in close proximity. Central veins are displaced and lose their normal relation to the lobules. The latter are reduced or increased in size and in many situations their boundaries cannot be defined. Fusion of hypereemic zones and of connective tissue bands produces partial or complete isolation of pseudolobular groups of liver cells which lack connection with a central vein.

Hemosiderosis, as shown by special stains for iron-containing pigment, is infrequent. No hemosiderin was found in 18 of the 30 cases in this study. The amount was minimal or slight in 7 cases and moderate in 5 cases. When present, the pigment was generally situated in both parenchymal and Kupffer cells in or at the periphery of the hypereemic zones. These findings favor the view that venous stasis of the central regions is usually not accompanied by significant hemorrhage. Some extravasation of erythrocytes occurs, but the cells may apparently remain intact for a considerable period.

Variation in morphology is characteristic of congestive cirrhosis. Necrosis of parenchyma varies in degree in the brownish red retracted zones. Fibrosis is prominent in the latter regions and slight or absent in the regenerative foci. The connective tissue varies in amount and location. Portal fibrosis, mixed portal and central fibrosis, and pure central fibrosis may be intermingled in nearby situations.

The gross and microscopic appearance of the liver is essentially similar for the various etiologic types of heart disease. However, in this study a tendency was noted for greater prominence of portal fibrosis in lesions accompanying constrictive pericarditis and hypertension than in those associated with rheumatic heart disease.

**Pathogenesis.** Degeneration and necrosis of hepatic parenchyma in chronic passive hyperemia occur mainly in the central zones. Although the lesion is sometimes referred to as pressure atrophy and necrosis, direct compression of cells by dilated sinusoids is not significant.<sup>4,9</sup> The changes are probably due to the prolonged anoxia which accompanies venous stasis and is most severe in the central regions.<sup>4,8,9,12</sup> The primary injurious factor is the passive hyperemia itself.<sup>1</sup> That infectious or other injurious agents<sup>10,15</sup> unrelated to venous stasis commonly play an additional rôle is not proven, even though impairment of circulation may predispose the liver to such action.<sup>3</sup> No such agents could be established in this study. Marked hepatic necrosis has been produced experimentally under conditions which apparently eliminate toxic or infectious factors.<sup>4,16</sup>

Degeneration and necrosis of parenchyma are followed by thickening and condensation of the stroma and finally by proliferation of fibrous connective tissue. The latter may represent the reaction of the stroma to anoxemia or possibly to injurious products released by passive hyperemia. Katzin *et al.*<sup>7</sup> found an increase in incidence and severity of hepatic fibrosis with increasing duration of congestive heart failure. Fibrosis frequently accompanies atrophy of parenchyma in any organ, and Karsner<sup>6</sup> points out that this is true of the cellular atrophy caused by prolonged passive hyperemia. As cells are injured and destroyed, the products of disintegration stimulate a low grade of inflammation that results in fibrosis. Moreover, the continued presence of edema may incite fibrosis.

In the experimental lesions produced in dogs by compression of the inferior vena cava, Zimmerman and Hillsmann<sup>18</sup> found early necrosis of liver cells, Kupffer cells, and periblobular connective tissue. There was active proliferation of fibrous tissue. However, the fibrosis was purely central and failed to increase in extent after 85 days. The authors observe that the rapid course and progressive nature of the lesion in these animals differ from patients with congestive heart failure who often show periods of arrest or improvement.

Some doubt exists as to whether congestive cirrhosis is a progressive lesion in the sense of persistent action of the injurious agent, or continuous progress of the lesion morphologically. Those cases in which the lesion appears to be progressive clinically, have usually been attributed to repeated episodes of congestive heart failure. Bolland

and Willis<sup>1</sup> even doubt that fibrosis can occur in the liver except in periods of recovery from decompensation. In our group of 30 patients, 20 gave a history of repeated attacks of failure, while 10 showed progressive failure without apparent remission. Nevertheless the latter may have had intervals of improvement with relief of hepatic venous stasis. It appears likely that recurrent episodes of decompensation promote the development of congestive cirrhosis.<sup>1</sup> However, this does not exclude production of the lesion as the result of severe, persistent venous stasis. In Zimmerman and Hillman's animals, the lesions of the liver, including the fibrosis, were steadily progressive for 85 days. Bolton's work suggests that failure to extend beyond this period may be due to establishment of collateral anastomoses.<sup>4</sup> Persistent mechanical interference with venous outflow from the liver, induced by constricting the inferior vena cava, is comparable in man to chronic constrictive pericarditis. The latter produces continuous and severe venous stasis of the liver. The development of congestive cirrhosis in such cases, as in 5 of the 8 patients in this study with constrictive pericarditis, is highly suggestive of a progressive process.

**Comparison With Laennec's Cirrhosis.** Congestive cirrhosis does not develop the extreme structural distortion and the extensive fibrosis and regeneration observed in the Laennec type. Moreover, the distribution of the lesion through the organ lacks the uniformity of the Laennec type. In the latter the causative agents produce severe, diffuse injury to parenchyma and mesenchymal framework. Congestive cirrhosis results from prolonged venous stasis and the injurious effect on the liver is less intense. Lack of uniform distribution through the organ is probably due to difference in degree of stasis in the radicles of the hepatic vein.

The morphologic distinction between well-developed Laennec's and congestive cirrhosis is simple. Differentiation of the early stage of Laennec's cirrhosis from the congestive lesion may be difficult. Microscopically, the portal fibrosis occurring in each form is indistinguishable. Central fibrosis is confined to patients with congestive heart failure.<sup>7</sup> Even when the fibrosis is chiefly portal in location, the congestive form usually shows central hypereimia with atrophy or necrosis of parenchyma. Moreover, in congestive cirrhosis the nodules of liver parenchyma are irregular in shape and show anastomosis, while those of Laennec's cirrhosis tend to be round, circumscribed, and isolated. Should early Laennec's cirrhosis be complicated by congestive heart failure, distinction may be impossible.

Some observers doubt that the term cirrhosis can be properly applied to livers the seat of chronic passive hyperemia. The main objections are that the fibrosis represents chiefly condensation of stroma and that the architecture is altered in a false rather than real sense, with limitation of nodularity to the region of sublobular veins. Lambert and Allison<sup>8</sup> held that chronic passive hyperemia does not produce cirrhosis of the usual portal or nodular type.

In this study, proliferation of connective tissue and distortion of

architectural pattern were the diagnostic criteria. Fibrosis was moderately diffuse, was active as indicated by the presence of fibroblasts, led to the formation of anastomosing collagenous bands, and was distinctive from mere condensation of stroma. The term was not applied to livers with focal fibrosis, nor to lesions principally subcapsular in position or confined to the lower portions of the two main lobes. Alteration of structural pattern was indicated by displacement of veins, isolation of nodular groups of liver cells, and frequent loss of identity of original lobular units.

The frequency of congestive cirrhosis depends on the criteria used for diagnosis and their interpretation. Accordingly, the lesion has been held to be non-existent,<sup>8,11</sup> rare,<sup>1</sup> or moderately frequent.<sup>6</sup> A liberal view of the concept of cirrhosis would permit inclusion of a congestive form of the disease. The term congestive cirrhosis is useful from a morphologic standpoint. There is, however, the reservation that the lesion is much less severe and less uniformly distributed through the organ than is Laennec's cirrhosis.

**Clinical Diagnosis.** A clinical diagnosis of congestive cirrhosis was made in 3 of the 30 cases in our study. This diagnosis is generally inaccurate because congestive cirrhosis as a group shows no distinctive features in comparison with organs the seat of advanced passive hyperemia. Apparent increase in size of the liver, ascites, jaundice, and palpable spleen are not adequate criteria; they are the result of decompensation and occur in both groups. The changes in stroma and vascular bed of the liver in congestive cirrhosis are not sufficient to produce portal obstruction. Blood protein levels are similar in the two groups and are generally reduced—a common finding in cardiac decompensation.<sup>14</sup> No investigation of liver function was made in our cases, but the studies of Boland and Willis<sup>1</sup> indicate that no significant differences are encountered in congestive cirrhotic and non-cirrhotic livers. Functional impairment appears to be an index of parenchymal atrophy and necrosis rather than cirrhosis. Marked decrease in function is rare.

Perhaps the best clues as to the presence of congestive cirrhosis clinically are obtained from the patient's history and the causative type of heart disease. The development of cirrhosis is favored by long-standing failure, especially with numerous episodes of decompensation. The lesion frequently accompanies chronic constrictive pericarditis and is relatively common in rheumatic heart disease. Garvin found that rheumatic and hypertensive heart disease were the types most commonly associated with cirrhosis.<sup>5</sup> His study included no cases of constrictive pericarditis.

**Summary and Conclusions.** Livers the seat of prolonged and advanced passive hyperemia, due to heart failure, sometimes show diffuse fibrosis and alteration of architectural pattern. These may properly be designated congestive cirrhosis. However, the degree of fibrosis and distortion of architectural pattern are considerably less than in well-developed Laennec's cirrhosis.

The main etiologic factor is prolonged and severe hepatic venous

stasis. Repeated episodes of decompensation favor the development of the lesion. The most severe degree of fibrosis and architectural change occurred in patients with chronic constrictive pericarditis. The continual venous stasis in such cases suggests that the cirrhosis develops as a progressive process. Congestive cirrhosis is relatively common in patients with rheumatic heart disease, both in those with mitral stenosis or with combined valvular lesions. It is less frequent in hypertensive patients and is uncommon or rare in other etiologic forms of heart disease. The clinical aspects of congestive cirrhosis do not provide adequate data for antemortem diagnosis.

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## ANNULAR PANCREAS

## A TABULATION OF THE RECENT LITERATURE AND REPORT OF A CASE

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The anomalous condition known as annular pancreas is reputedly rare, there having been less than 50 cases reported up to 1943. McNaught<sup>1</sup> was able to find 40 cases in the period from 1918 to 1932 and reported them collectively in 1933. It is apparent that in the intervening decade this condition made more frequent appearances in the literature. In 1935 McNaught<sup>2</sup> reported 3 additional cases which he had found in the literature and added 1 more of his own. More recently 2 others have appeared, one by Cunningham,<sup>3</sup> the other by Truelsen.<sup>10</sup> The pertinent facts of these additional cases and of the

case reported below are listed in Table 1. This table, together with that of McNaught,<sup>5</sup> completes the tabulation of the entire series of 47 cases.

The increasing frequency of case reports on annular pancreas suggests that the true incidence is probably higher than the available figures indicate. This contention is supported by the fact that within 14 years 3 cases of this anomaly have been found in the autopsy material at Receiving Hospital. From the time that Brines<sup>1,2</sup> encountered the 1st case until the 3rd case was found there were 7095 autopsies. This makes an incidence of 0.0412%, approximately 1 in every 2500 autopsies.

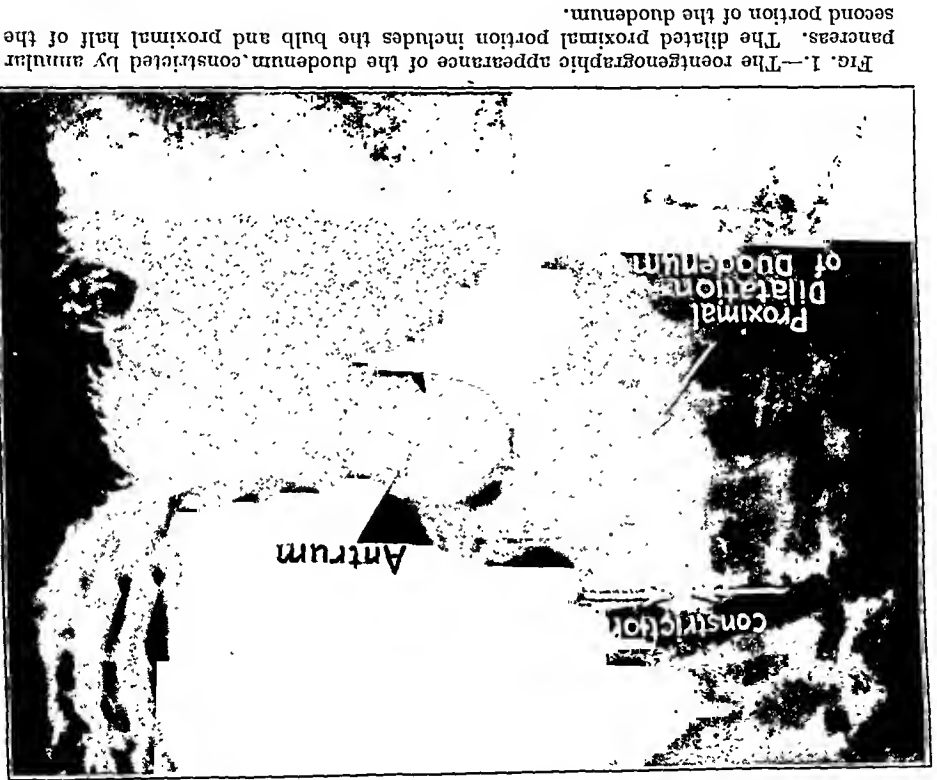


Fig. 1.—The roentgenographic appearance of the duodenum, constricted by annular pancreas. The dilated proximal portion includes the bulb and proximal half of the second portion of the duodenum.

The 2 early cases in the series at Receiving Hospital were complicated by gastro-intestinal lesions, the first by acute hemorrhagic pancreatitis and the second by peptic ulcer. Further details are not included.

**Case Abstract.** The last of the series was a white male, age 62, who had previously been under observation for depressive psychosis. He was admitted for the last time on October 27, 1942, because of his mental state. The history from the relatives revealed that he had been vomiting several times daily for the past 6 months. Physical examination showed emaciation, a normal chest and abdomen, external hemorrhoids, and well healed amputations of the great toes. By Roentgen ray an abnormality of the duodenum was demonstrated which was interpreted as a diverticulum (Fig. 1). The patient was put on a medical régime in an effort to improve his condition so that an explor-

atory laparotomy could be done. He failed to respond and died on December 4, 1942.

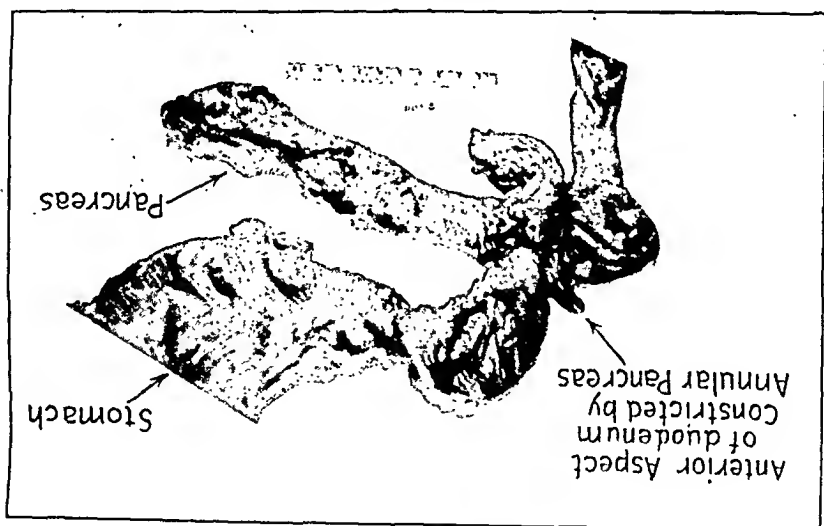


Fig. 2.—The gross specimen showing the dilation of the duodenum above and below the annular pancreas.

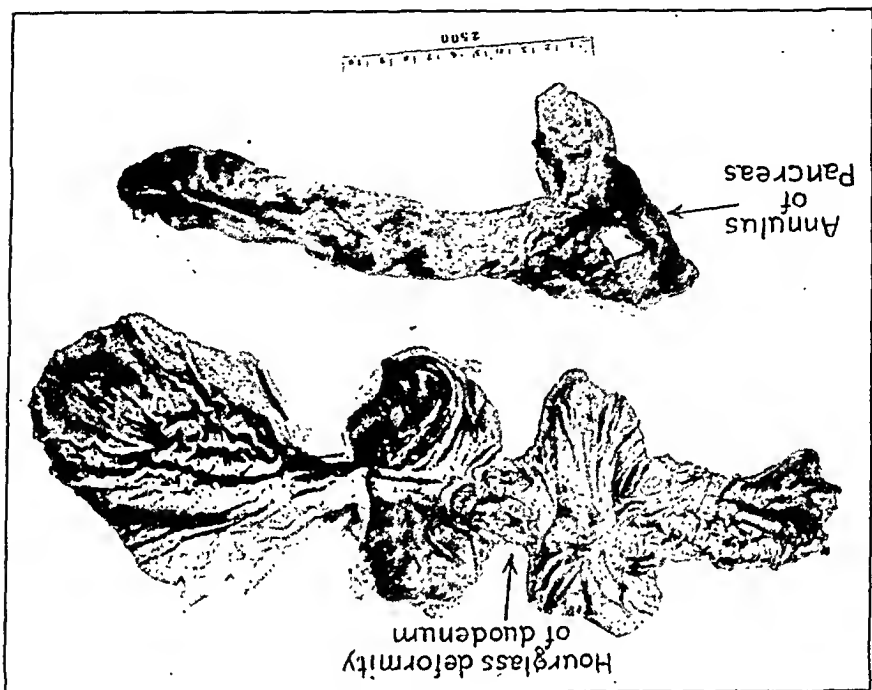


Fig. 3.—The dissected specimen showing the complete ring of pancreatic tissue.

**AUTOPSY.** An annular pancreas was found, associated with an "hour-glass" deformity of the duodenum and dilatation of the stomach (Fig. 2). In addition, there was an abnormal convolutional pattern in the cerebral cortex, enlarge-



ment of the ventricular spaces, small cavity formation in the globus pallidus, and chromophobe adenoma of the pituitary.\*

The ring of pancreatic tissue was complete (Fig. 3). However, on the posterior aspect of the ring near the head of the pancreas the tissue was attenuated, and loose, fibrous tissue composed part of the diameter of the ring at this level. Injection of the duct system in a manner similar to that suggested by McNaught<sup>1</sup> resulted in a failure to demonstrate any connection between the main pancreatic duct and that which, as proven by microscopic section, was present in the annular portion of the pancreas (Fig. 4).



Fig. 4.—The injected pancreas. The main duct system is completely injected. The duct of the annular portion remains uninjected and is apparently entirely separate.

This finding is in accordance with the experience of Cunningham,<sup>4</sup> who in his specimen was able to inject the annular duct system separately. Unfortunately we were unable to accomplish separate injection because of our inability to cannulate the extremely small duct in the annulus of the specimen. However, Schlesinger<sup>5</sup> demonstrated that his injection mass would penetrate any vessel with a caliber

\* The examination of the brain was done by Dr. Gabriel Steiner, Chief of the Division of Neuropathology, Wayne University College of Medicine.

Associated congenital abnormality	Age	Sex	Signs and symptoms	Duodenum		Double hiatus of the diaphragm with visceral displacement	Incision and transverse suture	Malformation abdominal viscera	Horseshoe kidney	0	0	Cerebral malformation
				In Con- striction	Dilatation							
	1927	M	9 mo. fetus	Second	+	Not demon-	0					
	1931	F	Pain in right hypochondrium, fulness, tenderness	Second	+	Not demon- strated	Incision and transverse suture	0				
	Reitano <sup>7</sup> 1932	M	5 mo. fetus, acholic	Third*	+	Not demon-	0	Malformation abdominal viscera	Horseshoe kidney	0		
	McNaught <sup>14</sup> 1933	M	None related to pancreas	Second	0	Opened into main duct	0					
	Cunningham <sup>1</sup> 1940	M	0	Second	0	Duct separated; opened into common bile duct	0					
	Truesen <sup>10</sup> 1940	M	Epigastric pain, fulness, vomiting	Third	+	Not demon-	Duodenor-	0				
	Stofer	M	Vomiting	Second	+	Separate	0					

\* Not complete.  
† Markedly thickened wall.

TABLE 1.—ANALYSIS OF CASES REPORTED SINCE MCNAUGHT'S LIST (1933)

Associated congenital abnormality	Operation	Age	Sex	Signs and symptoms	Involvement in Constriction	Duodenum		Duct in ring	Double hiatus of the phragm with visceral displacement	Zeck 1931	Reitano? 1932	McNaught? 1935	Cunningham, 1940	Truelsen? 1940	Stofer
						Dilatation	Duct in ring								
		9 mo.	M	0	Second	+	+	Not demonstrated	0						
		27	F	Pain in right hypochondrium, fulness, tenderness	Second	+	+	Not demonstrated	Incision and transverse suture	0					
		5 mo.	M	Icterus, acholic feces	Third	+	None	Not demonstrated	0	Malformation abdominal viscera	Horseshoe kidney	0			
		70	M	None related to pancreas	Second	0	0	Opened into main duct	0						
		65	M	0	Second	0	0	Duct separated; opened into common bile duct	0						
		35	M	Epigastric pain, fulness, vomiting	Third	+	+	Not demonstrated	Duodenorhaphy and gastroenterostomy	0					
		62	M	Vomiting	Second	+	+	Separate	0	Cerebral malformation					

↑ Markedly thickened wall.

That other congenital malformations are frequently associated with annular pancreas is well known. In the complete series this association occurs in 12 of the 47 cases (25.5%). Most of these abnormalities are in the gastro-intestinal tract. This is the first case in which the associated abnormality is in the central nervous system.

ulm has been made. Greater consideration of the possibility of annular pancreas is justified by this experience. Summary. The 47th case of annular pancreas is reported, and the incidence in a large series of autopsies is established. Features of the case relative to pathogenesis, associated anomalies, and the clinical diagnosis are briefly discussed.

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## THE ELECTROCARDIOGRAM AND THE "TWO-STEP" EXERCISE

### A TEST OF CARDIAC FUNCTION AND CORONARY INSUFFICIENCY\*†

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In many cases of coronary artery disease with angina pectoris the physical examination, the size of the heart and cardiac pulsations, as seen in the Roentgen ray film and fluoroscopically, and the electrocardiogram are normal. There is thus no objective evidence to confirm the diagnosis of coronary sclerosis. In valvular disease patients often complain of symptoms such as dyspnea and pain although the physical examination reveals no evidence of heart failure. In both of these types of cases a functional test, *i. e.*, one that obtains the response of the heart to effort, may be of great value both in diagnosis and the evaluation of functional capacity. Beginning in 1925 one of us developed a test utilizing the response of the blood pressure and pulse rate to a standard "two-step" exercise. This measurement of vaso-motor response was found to be a practical indication of circulatory fitness. Later the ECG findings following this standard "two-step" exercise.

\* Read in greater part before the joint session of the Section of Medicine, New York Academy of Medicine, and the New York Heart Association, New York, Jan. 21, 1943. † Approved for publication by the Division of Publications, Bureau of Medicine and Surgery, U. S. Navy. The assertions herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

cise were used to reveal the state of the coronary circulation.<sup>10,12,13,14,15</sup> If coronary insufficiency existed, characteristic changes occurred in the ECG. No changes appeared in the presence of adequate coronary circulation. We are reporting results which for the first time definitely show that the abnormal changes in the ECG following the (Master) "two-step" exercise are due to the lack of oxygen supply to the heart muscle, i. e., coronary insufficiency. Whenever ECG changes appeared after the "two-step" (e. g., RST depressions or T-wave inversions) they were reproduced exactly in the same subject by his breathing a low oxygen concentration, specifically 10% oxygen. When no ECG abnormalities appeared after the "two-step" none appeared on breathing 10% oxygen.

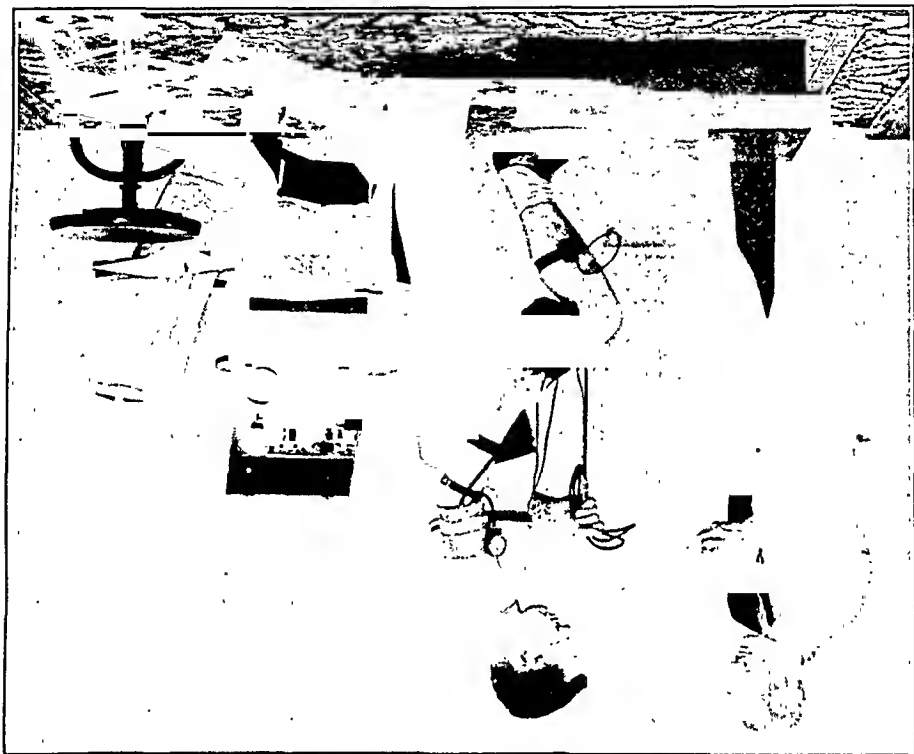


Fig. 1.—The set-up with standard "2-steps" each 9 inches high, an ECG machine, Adironack chair with wide arms, technician or physician with stop watch in hand, person to be tested with electrodes strapped on and blood pressure cuff and gauge bound around arm.

**Procedure of the "Electrocardiogram and Two-step."** The electrodes are attached to the patient and the blood pressure cuff placed on his arm (Fig. 1). He is then weighed. His name and age are written down and the number of trips he is to make is obtained from the table (Table I). For example, H.A.K., a man of 55, weighing 164 pounds calls for 19 trips. The electrocardiogram is recorded. Then the blood pressure and pulse rate are taken alternately until they are stabilized, that is, until the last 2 blood pressures and the last 2 pulses

do not differ more than 2 points. (The pulse is taken for 15 seconds and multiplied by 4.) The resting figures are obtained usually in but a few minutes. The technician or physician then gives an actual demonstration by climbing over the steps 2 or 3 times. The examinee at a given signal walks up one side of the steps and down the other, always turning to the same wall or side of the room before each ascent. The candidate makes a climb only when he receives the count and the required number of ascents is completed in  $1\frac{1}{2}$  minutes. If the candidate climbs too slowly the counting is made more

TABLE 1.—NUMBER OF TRIPS TO BE PERFORMED IN THE "TWO-STEP" TEST, ARRANGED ACCORDING TO SEX, AGE AND WEIGHT

Weight (lbs.)	Age (yrs.)																		
	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69						

## Males

40-49	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
50-59	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
60-69	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
70-79	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
80-89	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
90-99	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
100-109	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
110-119	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
120-129	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
130-139	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
140-149	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
150-159	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
160-169	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
170-179	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
180-189	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
190-199	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
200-209	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
210-219	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
220-229	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

## Females

40-49	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
50-59	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
60-69	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
70-79	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
80-89	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
90-99	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
100-109	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
110-119	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
120-129	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
130-139	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
140-149	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
150-159	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
160-169	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
170-179	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
180-189	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
190-199	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
200-209	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
210-219	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
220-229	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14

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rapid and if the climbing is too fast the counting is made more deliberate. A stop watch or an electric clock with a large second hand is used. At cessation of the exercise the candidate sits down and the 4 leads of the ECG are recorded immediately. At about  $1\frac{1}{2}$  to  $1\frac{3}{4}$  minutes later the blood pressure is taken. One should try to finish this reading just about 2 minutes after the completion of the exercise. The ECG is now repeated and the 2 minute pulse rate obtained from this tracing or by radial pulse count. The ECG is repeated at 2 minute intervals until 10 minutes after exercise cessation of exercise. Both the blood pressure and pulse rate 2 minutes after exercise should be within 10 points

of resting levels. Anything above 10 points in the blood pressure and pulse rate is considered abnormal. The following is an illustration:

H.A.K.: Coronary heart disease, age 55, weight 164 pounds; required number of trips 19.

Blood pressure		Pulse	
Resting figures			
114/82		86	
110/80		82	
108/80		82	
132/80		94	
Two minutes after exercise			

Conclusion: Lag in return both of systolic blood pressure and pulse rate following "two-step" exercise.

The number of climbs on the steps which the patient performs is obtained from previously prepared tables for normals based on age, sex and weight (Table I). The tables<sup>15</sup> are the results of more than 2000 tests performed at Cornell University Medical College, 1925-29, on normal persons of all ages and both sexes. They were constructed on the basis that the blood pressure and pulse rate should return to the resting levels 2 minutes after cessation of exercise. The 9 inch size of steps, the two together adding up to 1½ feet in height, and the 1½ minute duration of exercise were both chosen after careful and deliberate experimentation and their value confirmed by years of experience. The response of the blood pressure and pulse rate and the ECG are combined in one procedure.

The examinee turns to the same wall or same side of the room, actually a change in direction at each turn, to prevent giddiness which would result in artificial changes in blood pressure and pulse rate. Placing the steps near or against a wall gives a patient a sense of security. In fact, the examiner, further to reassure such a person, can support him by holding his arm and guiding him over the steps provided he exerts no vertical lift.

A large electric clock with a large sweeping second hand may be preferable to a stop watch as it is seen clearly by all. One technician suffices for the test but, if it is desired, one technician or physician records the blood pressure and the pulse and another technician takes the ECG. In this case the one large electric clock is seen clearly by both workers. If the clock is used instead of the stop watch it is practical to start the exercise on a ½ minute mark so that the exercise of 1½ minutes will be completed when the second hand has made a complete revolution, *i. e.*, reached the 12 o'clock hour. If a stop watch is used this is not necessary for at the completion of the exercise the mechanism can be started anew.

Frequently the ECG changes occur only immediately after exercise and so it is important to be able to obtain the 4 leads quickly. We have taken all 4 leads in as short a period of time as 15 to 30 seconds.\* Enough heart beats should be registered to have at least 2 or 3 on the same horizontal line as otherwise artificial changes in the RST level occur. At the present time we repeat the ECG at 4 and 10 minute intervals after cessation of exercise, since occasionally a change becomes apparent only late.

Using the PR interval of the ECG as the control level, a depression of the RST segment over 0.5 mm. in any lead is a positive result and does not occur in healthy persons. In other words, the level of the string just following the QRS complex is compared with that immediately preceding this complex.<sup>13</sup> A change from an upright T wave to an isoelectric (flat) or inverted T wave is also an abnormal response; so, too, is a change in the other direction, *i. e.*, a negative T wave to a

\* The new "instomatic" type of small portable ECG of the Sanborn Instrument Company has been found to be particularly useful since a change from one lead to another can be made instantly without the necessity of waiting for the light beam to come to rest.

positive. Occasionally premature beats or some more significant arrhythmia, widening of the QRS, large Q waves, prolongation of the PR interval or heart block may appear. As will be seen later, a control ECG with RST depressions and T-wave inversions indicates coronary insufficiency to begin with and hence the exercise test is unnecessary. The combination of the blood pressure and pulse response and the ECG in one procedure saves time as both tests are performed simultaneously. The criteria of abnormality are conservative and have been worked out after experience in many normals.<sup>13</sup>

To obtain evidence that the ECG changes after the "two-step" were caused by anoxemia of the heart muscle, the 10% oxygen anoxemia test was made on every normal control and patient who performed the "electrocardiogram and two-step." Anoxemia, that is, the breathing of oxygen below atmospheric content, as a test for heart function has been suggested for years but Levy and his co-workers<sup>14</sup> first worked out a definite technique.\* After the mask was adjusted and made comfortable the patient breathed through it for a few minutes in order to become accustomed to it. Then a control ECG was taken before breathing 10% oxygen. The ECG was repeated every 5 minutes while breathing the mixture of low oxygen for a total duration of 20 minutes unless circulatory collapse, sudden significant fall of blood pressure, syncope, etc., occurred.

**Material.** The subjects were 84 Navy personnel between the ages of 17 and 66 (Tables 2 and 3). There were candidates for the Navy and patients with coronary heart disease, hypertension, valvular heart disease, effort syndrome, and a miscellaneous group, i. e., chest deformities, congenital heart disease, etc. Thirty-three healthy men served as normal controls (Table 2). The work was done at the National Naval Medical Center, Bethesda, Md.

**Results. NORMALS.** There were no positive ECG changes following the "two-step" in the 33 normal individuals (Fig. 2). In other words, there were no flattening or inversion of a normal T wave (or the converse) and no depression of the RST segment relative to the PR level of more than 0.5 mm. One youngster, U.T.S. (Case 20), whom we had been using as a normal control for certain experiments, showed depressed RST but this was explained when a Roentgen ray film disclosed that he was an unsuspected case of virus pneumonia. On recovery his "electrocardiogram after the two-step" became normal. A severe upper respiratory infection, gastro-enteritis (Case 22), and occasionally lack of rest and sleep (Case 18), produced changes. The 10% oxygen test caused similar changes. This test is much more severe than the "two-step." For example, it produced almost immediate peripheral vascular collapse in the unrecognized case of virus pneumonia whereas the "two-step" had only tired the boy moderately.

\* Ten per cent oxygen was obtained from either the Experimental Diving Unit, Navy Yard, Washington, D. C., or the Ohio Chemical and Manufacturing Company, Cleveland, Ohio. A simple rubber face mask covering nose and mouth was used with 2 pin flutter valves, 1 for inhalation of the 10% oxygen and the other for expiration of carbon dioxide. In other words, there was no rebreathing. Both valves were sensitive and offered negligible resistance to the passage of air.

TABLE 2.—"ELECTROCARDIOGRAM AFTER 2-STEP" AND 10% OXYGEN TEST IN

Electrocardiogram

Changes

No.	Name	Age	Before test	2-Step	10% oxygen	Remarks
1	D.M.B.	28	Neg.	None	None	
2	H.A.B.	20	"	"	"	
3	F.C.	19	"	"	"	
4	A.S.F.	27	"	"	"	
5	D.K.	18½	"	"	"	
6	Z.L.	26	"	"	"	
7	A.M.A.	46½	"	"	"	
8	R.P.M.	20	"	"	"	
9	S.N.	26	"	"	"	
10	S.R.	26	"	"	"	
11	J.S.	37	"	"	"	
12	J.M.S.	27	"	"	"	
13	J.W.T.	25	"	"	"	
14	R.W.T.	23	"	"	"	
15	R.C.W.	25	"	"	"	
16	W.C.B.	20	"	"	"	
17	H.F.H.	23	"	"	"	
18	M.L.T.	26	"	"	RST 1-2-3	O <sub>2</sub> test performed when man sleep- ess and fatigued
19	J.R.Mc	34	"	"	None	
20	U.L.S.	20	"	T 2-3	T 2-3	Pneumonia
21	W.O.P.	26	"	None	None	Recovered
22	J.B.W.	20	"	T 2	"	Gastro-enteritis
23	M.S.A.	21	"	None	"	
24	H.C.	19	"	"	"	
25	G.C.	17	"	"	"	
26	A.V.E.	19	"	"	T 2; T 3	
27	G.L.G.	19	"	"	None	
28	A.P.H.	20	"	"	"	
29	G.W.L.	28	"	"	"	
30	M.E.Mc.	17	"	"	"	
31	E.G.R.	17	"	"	"	
32	H.T.	18	"	"	"	O <sub>2</sub> stopped at 10 min. unresponsive
33	H.A.T.	21	"	"	"	

TABLE 3.—"ELECTROCARDIOGRAM AFTER 2-STEP" AND 10% OXYGEN TEST IN

Coronary Heart Disease

34	W.G.B.	66	Ventr. prem. beats; T 2, T 3; RST 2	T 2, T 3	T 2, T 3, RST 1-T 4	
35	J.A.C.	59	Neg.	T 2, T 3	T 2, T 3	
36	D.M.C.	49	T 1-2-3-4 inverted	T 2-3-4	T 1	
37	E.D.C.	44	Neg.	None	None	
38	D.A.D.	35	140/min. RST 1-2-3, depressed, T 1-2-3	"	"	
39	A.J.D.	60	QRS 0.11, Q 1-4, T 1-4 inv. semi-inv.	T 1-2-4	T 1-4	
40	G.A.G.	46	Neg.	None	None	
41	J.H.G.	63	QRS low, Q 4, T 1 inv.	T 1	T 1	O <sub>2</sub> stopped at 6 min.; collapsed
42	R.S.H.	60	PR = 0.23	None	Nodal rhythm	O <sub>2</sub> stopped at 5 min.; nausea, dyspnea
43	P.O.H.	44	Neg.	"	None	
44	H.A.K.	55	"	RST 1-2-3-4, T 1-2-3-4	RST 1-2-3-4, T 1-2-3-4	
45	R.S.K.	59	Neg., P 2-3 inv.	P 2-3, Q 4	P 2-3	
46	P.S.L.	57	T 1 flat	T 1-2	T 1	
47	H.P.Mc.	45	Q 3, T 2-3 inv.	T 2	T 2	
48	O.P.	41	QRS notched, Q 2-3	None	None	
49	W.H.S.	48	Neg.	T 1-2	None	O <sub>2</sub> stopped at 17 min.; collapsed



TABLE 3.—(Continued)

No.	Name	Age	Before test			2-Step		Remarks
			QRS 0.12	Neg.	QRS 0.11, Q 2-3, T 2-3, T 1-2 inv., Q 3	RST 1, T 1	RST 1-2-4, T 1-2-4, T 1-2-4	
53	J.E.T.	44	T 1-2 inv., Q 3	T 1	T 1	T 1	T 1	O <sub>2</sub> stopped at 15 min.; collapsed
52	H.S.	63	QRS 0.11, Q 2-3, T 2-3, T 1-2 inv., Q 3	None	None	None	None	O <sub>2</sub> stopped at 15 min.; collapsed
51	Mrs. C.P.S.	63	Neg.	None	None	None	None	
50	L.P.S.	51	QRS 0.12	None	None	RST 1, T 1	RST 1-2-4, T 1-2-4, T 1-2-4	
Valvular Heart Disease								
54	H.L.A.	29	Neg	None	None	None	None	O <sub>2</sub> stopped at 11 min.; mild shock
55	V.L.A.	20	P 2-3 notched, QRS high, Q 2-3, RST 2-3, T 2-3 inv.	None	None	None	None	
56	L.A.C.	25	T 2-3 inv.	T 2	T 2	T 2-3	T 2-3	
57	W.P.F.	23	T 2 flat	T 2-3	T 2-3	T 2-3	T 2-3	
58	H.M.G.	27	QRS 0.12, right axis deviation	RST 2, T 2	RST 2, T 2	T 2	T 2	
59	H.T.H.	37	Neg.	RST 2-3	RST 2-3	RST 2-3	RST 2-3	
60	D.C.L.	23	"	None	None	None	None	
61	H.F.A.L.	39	Neg, P 2 notched	"	"	"	"	O <sub>2</sub> stopped at 15 min.; peripheral failure
62	Mrs. C.L.	22	105 per min., T 2 flat, T 2 inv.	T 2	T 2	T 2	T 2	
63	M.E.M.C.	24	Neg.	None	None	None	None	
64	Mrs. C.S.	25	P 2-3, RST 2-3, T 2-3	RST 2-3	RST 2-3	T 1-2-3-4	T 1-2-3-4	O <sub>2</sub> stopped at 15 min.; cyanosis
65	Mrs. L.T.S.	35	3 sec. inv., T 4 inv.	None	None	None	None	
66	E.M.T.	25	Neg.	RST 2-3, T 1-2-3-4	RST 2-3, T 1-2-3-4	RST 1-2, T 1-2-3-4	RST 1-2, T 1-2-3-4	
Hypertension								
67	L.A.	52	Neg, P 2 notched	None	None	None	None	O <sub>2</sub> stopped at 16 min.; B.P. 144/94
68	M.W.B.	42	Q 3	"	"	"	"	O <sub>2</sub> stopped at 7 min.; asleep, B.P. 160/120
69	J.H.S.D.	64	RST 1-2	"	"	"	"	B.P. 160/84
70	H.T.D.	62	Neg.	"	"	"	"	B.P. 170/120
71	J.R.E.	41	RST 2, T 1 inv.	"	"	"	"	B.P. 176/116
72	J.M.H.	42	Neg, 100 per min.	"	"	"	"	B.P. 170/118
73	T.J.K.	42	T 1-2-3 inv.	RST 2	RST 2	RST 2	RST 2	B.P. 190/116
Effort Syndrome								
74	A.G.B.	23	55 per min.	T 2	T 2	T 2	T 2	
75	L.S.M.	29	Neg, 100 per min.	T 2	T 2	T 2	T 2	
76	A.M.R.	43	" 100	"	"	"	"	O <sub>2</sub> stopped at 5 min.
77	E.M.T.	27	" 84	"	"	RST 2-3, T 1-2-3	RST 2, T 1-2-3-4	collapsed
78	D.A.D.	35	" 120	"	"	None	None	
79	J.J.B.	25	" 110	"	"	RST 2-4	RST 2-3-4, T 1-2-3	
80	O.L.B.	30	" 88	"	"	T 1	T 1	
81	J.W.C.	20	" 96	"	"	RST 2-3, T 2	RST 2-3	
Miscellaneous								
82	L.M.S.	21	45 per min.; many prem. vent. beats, T 1 flat	T 1	T 1	None	None	O <sub>2</sub> stopped at 15 min.; collapsed (bradycardia)
83	J.W.W.	34	105 per min., T 1-2-3-4 inv.	T 1	T 1	None	None	Comp. dilation, pulmonary artery funnel chest
84	Mrs. D.R.	24	Neg.	RST 2-3, T 2	RST 2-3, T 2	RST 2-3, T 2	RST 2-3, T 2	

CORONARY HEART DISEASE. There were 20 patients with coronary heart disease, 8 of whom had previously suffered coronary artery occlusion. The ages ranged from 35 to 66 years. Thirteen patients developed abnormal ECG after the "two-step" test and this evidence of coronary insufficiency was substantiated by the 10% oxygen test (Table 2).

In every patient in whom the "electrocardiogram after the two-step"

was normal the 10% oxygen test was normal, except possibly in Patient R.S.H. (Case 42) in whom a nodal rhythm appeared after breathing low oxygen. In this instance the 60 year old man collapsed after only 6 minutes of the diminished oxygen supply.

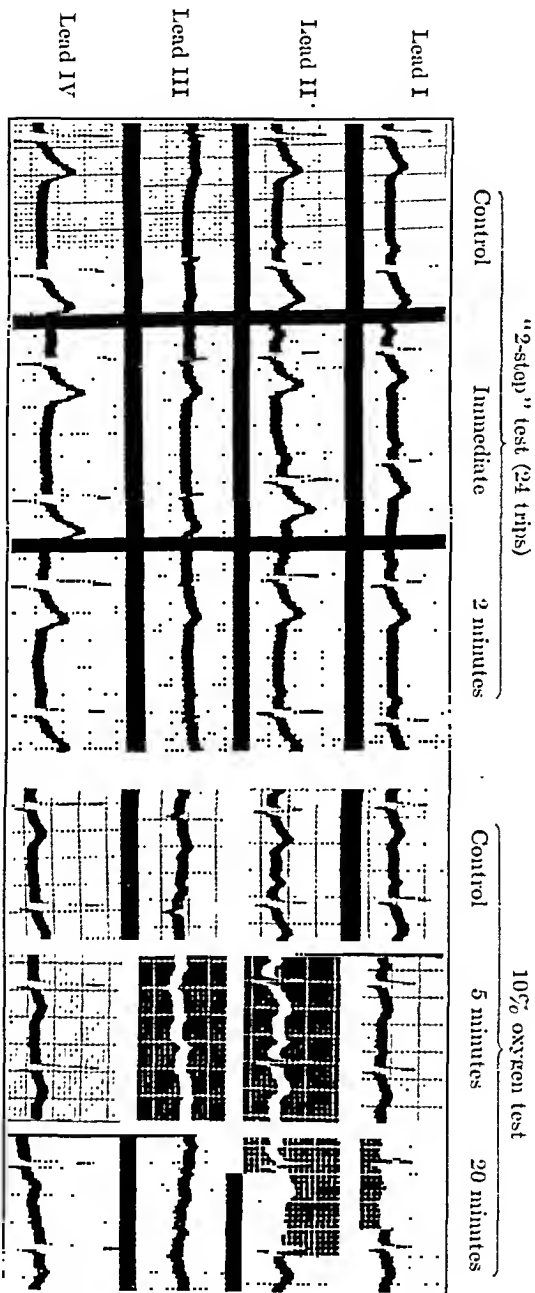


Fig. 2.—M.S.A., male, age 21. Normal subject. Control ECG negative and no change following the "2-stop" exercise. Similarly no change in the ECG on breathing 10% oxygen for 20 minutes.

Occasionally the results of the "two-step" were positive whereas the 10% oxygen was negative. Clinical experience proved the findings of the "two-step" to be correct. In Patient R.S.K. (Case 45) a

Q 4 appeared after the "two-step" and not after the 10% oxygen. This marine corps officer had suffered an acute coronary artery occlusion a few years before and was being reexamined for possible return to active duty. He was found unfit. Patient W.H.S. (Case 42) was examined because of an anginal syndrome. Physical examination and the control ECG were normal. However, the "electrocardiogram after two-step" exercise revealed significant T 1 and T 2 changes and so a diagnosis of coronary heart disease was made, although the 10% oxygen test was normal. That the result of the former test was truly indicative of coronary insufficiency was proven 3 months later when the man was admitted to the Naval Hospital because of severe chest pain with fever, leukocytosis and increased sedimentation rate. It is significant that the ECG in this attack was similar to that following the "two-step."

In 2 other patients clinical experience confirmed the value of the "electrocardiogram after the two-step" as a test of latent coronary insufficiency. The patients, H.A.K. (Case 44) (Fig. 3a) and L.P.S. (Case 50) first entered the Naval Hospital because of severe coronary heart attacks. Months later, after the ECG had returned to practically normal and there had been clinical improvement, the "electrocardiogram after two-step" was taken and found to be positive (Fig. 3, b). The ECG revealed changes similar to those seen in the actual heart attacks."

The control ECG of 7 of the patients with coronary heart disease were normal and 4 became definitely abnormal following the "two-step." Hence, the test is of particular value for detecting coronary insufficiency when it is latent.

Although many of the coronary heart patients were definitely ill, there was not one bad reaction to the "two-step" test. Patient H. S. (Case 52) had actually suffered a coronary occlusion 2 months before. He stood the "two-step" very well, yet when the 10% oxygen test was performed he became so completely exhausted that it was stopped before the time was up. Two other patients collapsed during the oxygen test, R.S.H. (Case 42), W.H.S. (Case 49), and, in one other, F.O.H. (Case 43), severe nausea and dyspnea appeared in 5 minutes. It is thus clear that the 10% oxygen is a severe task for moderately sick cardiac patients whereas the "two-step" is readily performed.

Summarizing these cases, the "electrocardiogram after the two-step test" is a measure of coronary insufficiency or anoxemia of the heart muscle as proven by the 10% oxygen test and as shown by long clinical experience. It is particularly useful when the control ECG is normal. The exercise is harmless.

**VALVULAR HEART DISEASE.** Thirteen patients suffering from chronic cardiac valvular disease, with mitral or aortic valve involvement (ages 20 to 39 years), were tested. There were 10 with mitral disease alone, 1 pure aortic, and 2 with combined lesions. Here, again, whenever the "electrocardiogram after the two-step" was abnormal the ECG in the 10% oxygen test confirmed this evidence of coronary insufficiency. This was true in 7 cases.

Two patients with valvular heart disease whose "electrocardiograms after the two-step" exercise were positive possessed normal control ECG. This again emphasizes the usefulness of this test for latent coronary insufficiency.

Not one of these 13 ambulatory patients was affected in any way by the "two-step" exercise but 3 suffered severe reactions in breathing 10% oxygen. In patients suffering from chronic cardiac valvular disease the 10% oxygen test requires a close watch.

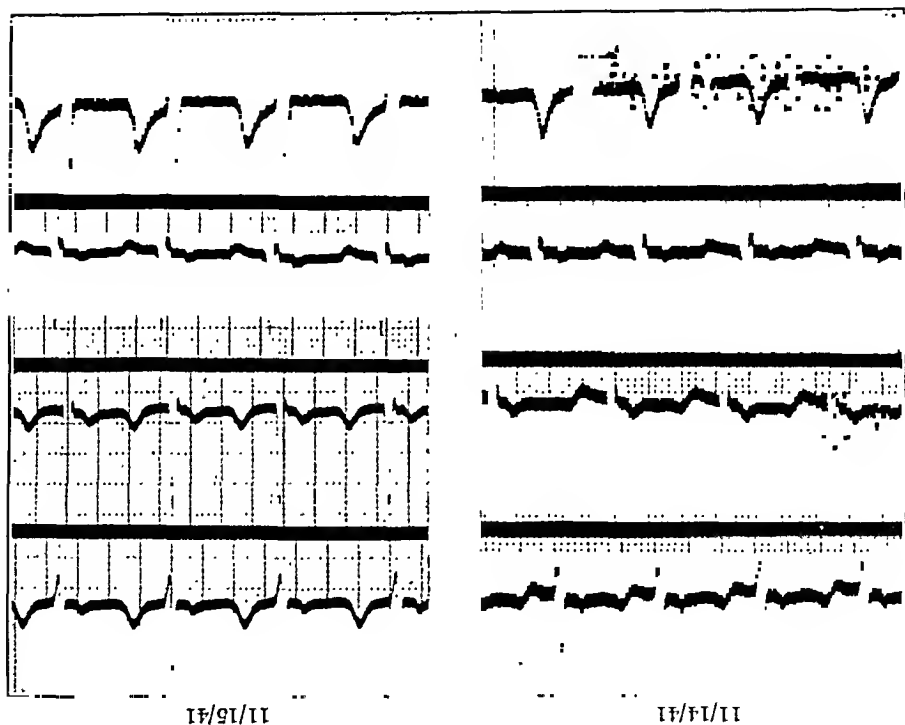


Fig. 3a.—H.A.K., age 54, U. S. N. Acute coronary insufficiency. Coronary heart disease. An attack of severe chest pain Nov. 14, 1941, for which immediate hospitalization was required. The ECG disclosed RST depressions and T-wave inversions in Leads I and II. By the next day, concomitant with the subsidence of the pain, the tracing had returned to normal.

It is to be expected that patients suffering from mitral stenosis frequently disclose coronary insufficiency following the "two-step," for in a "tight mitral" not enough blood reaches the left ventricle. Consequently, when this chamber contracts, only a small quantity of blood is ejected into the aorta and an insufficient quantity of blood enters the coronary arteries, resulting in myocardial anoxemia. The RST depressions and T-wave inversions are presumably the consequence of this muscle anoxemia. In aortic insufficiency a similar mechanism may be present, for, because of the regurgitation back to the left ventricle, an inadequate quantity of blood enters the coronary arteries.

In valvular heart disease the ordinary ECG is so frequently normal that the ECG after the "two-step" exercise is of particular value for testing for anoxemia of the heart muscle on effort.

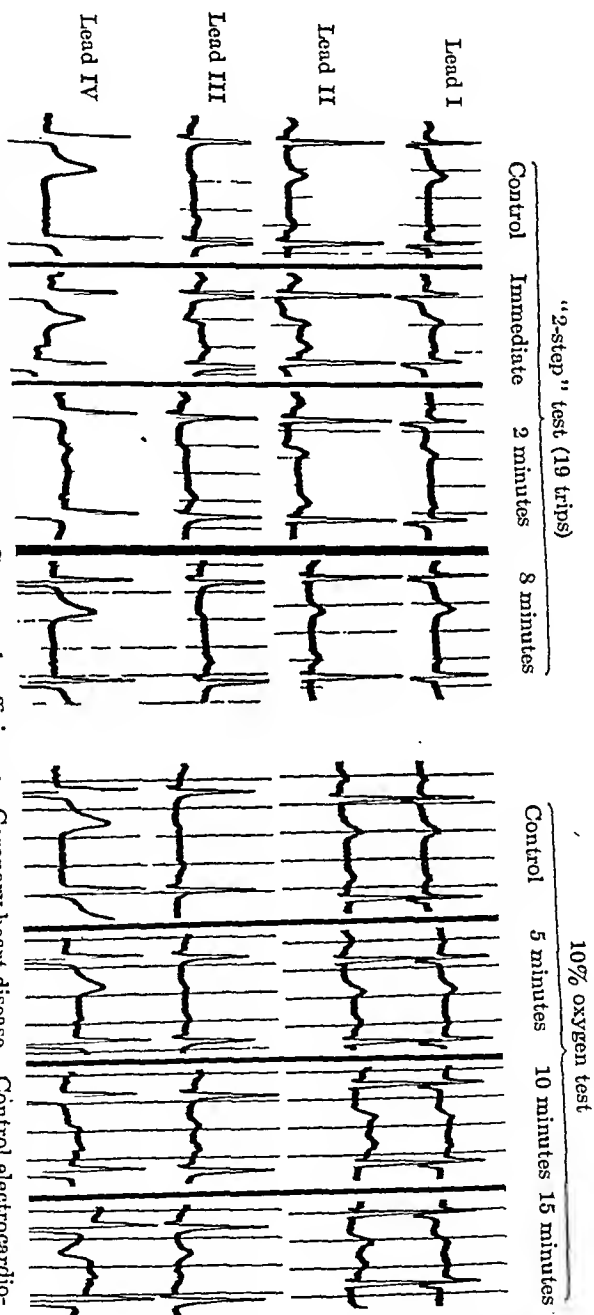


FIG. 36.—H. A. K., age 54, U. S. N. Coronary insufficiency. Coronary heart disease. Control electrocardiogram normal. Immediately following "2-step" exercise marked depression of the RST segment in all leads and inversion of T 2 and 3 occur. At 2 minutes, the T wave becomes semi-inverted. Return to normal in 8 minutes. After breathing 10% oxygen there were marked depressions of the RST segments and inversion of the T wave in Leads I, II and IV.

HYPERTENSION. There were 7 patients (41 to 64 years of age) with the diagnosis of "arterial hypertension" in whom both the "electrocardiogram after the two-step" and the 10% oxygen tests were performed. In this group also it was true that when the "two-step"

was negative there were no ECG changes on breathing 10% oxygen. In the one patient who demonstrated RST depressions in Lead II of the ECG after the exercise exactly similar results were obtained on breathing low oxygen. Whereas the "two-step" caused no ill-effects, the first 2 patients (Cases 67 and 68) could not finish the 20 minutes for the 10% "anoxemia" test.

One patient with hypertension showed positive changes after the test. However, 3 subjects, J.H.S.D. (Case 69), J.R.E. (Case 71) and T.J.K. (Case 73) revealed control ECG which indicated coronary insufficiency, *i. e.*, RST depressions or T-wave inversions, and so it is no wonder that there was no change after the exercise. [Similar observations were made in D.A.D. (Case 38), a man suffering with coronary heart disease, and in subject V.L.A. (Case 55), a man with valvular heart disease.]

**EFFORT SYNDROME OR NEUROCIRCULATORY ASTHENIA.** Both the "electrocardiogram after the two-step" and the 10% anoxemia test were performed in 8 cases of marked neurocirculatory asthenia or effort syndrome. Seven ECGs became abnormal after the "two-step." The anoxemia test gave similar results in all but 1 case (Case 76). (No comparison could really be made here for after only 5 minutes of breathing 10% oxygen the patient collapsed with all the evidence of peripheral vascular failure.)

We are of the opinion that the positive "electrocardiogram after the two-step" so often found in the effort syndrome patient is evidence that the small or hypoplastic heart sometimes found in such a person is constitutionally inadequate to carry on exertion. These people tire on effort because they have a diminished cardiac output<sup>7</sup> and the oxygen saturation of the blood is low<sup>8</sup> and because they develop anoxemia of the heart muscle as manifested by the "electrocardiogram following the two-step."<sup>10</sup> Of course the coronary arteries themselves may be entirely normal.

**MISCELLANEOUS CONDITIONS.** *Congenital Heart Disease.* Two patients, L.M.S. (Case 82) and J.W.W. (Case 83) with congenital dilatation of the pulmonary artery were examined and in both the "electrocardiogram after the two-step" was positive, but the 10% test was positive only in the first. Incidentally, this man collapsed during the inhalation of the oxygen.

*Chest Deformity.* One patient, D. R. (Case 84), a woman with a funnel-shaped depression, disclosed similar RST depressions and T-wave inversions in both the "electrocardiogram after the two-step" and the 10% anoxemia tests, proving that coronary insufficiency was present. Clinically, a low vital capacity and a low breath-holding time added confirmation to this explanation.

**Comment.** As previously remarked, it has been suspected for a long time that the RST depression and T-wave inversion following exercise were caused by anoxemia of the heart muscle, *i. e.*, the coronary circulation was not responsive to the demand of the heart muscle for oxygen.<sup>2,13,16</sup> We have definitely proved it. Our results show an uncanny correlation between the ECG following the "two-step"

exercise and that obtained after breathing diminished oxygen, *i. e.*, 10% oxygen.

It is interesting that 10% oxygen, rather than 11 or 9%, produces exactly the same changes in the ECG as the "two-step," exercise. We have experimented with oxygen from 8.5 to 12% and have found that 10% oxygen gave the same results as the "two-step," whereas anything less than this produced more marked ECG changes (and in addition was too dangerous for patients suffering from cardiac and pulmonary disease) and concentrations of oxygen higher than 10% did not produce sufficient anoxemia of the heart muscle.

The significance of exercise tests by earlier investigators was obscure because the amount of work performed by the individual being tested was not standardized. One investigator exercised the subject to fatigue, another only half as much. This is an important consideration because it is known that even a normal man may show ECG changes if allowed to exercise long enough.<sup>13</sup> Hence, the "two-step" exercise has been standardized according to sex, age and weight. This, too, is the first time that the ECG changes have been related to a definite amount of work which will not produce ECG abnormalities in a normal person.

Riseman<sup>16</sup> and his co-workers used the "two-step" exercise and concluded that it was not of value. However, they regarded any change in the RST as significant, not realizing that the RST segment must be depressed below the isoelectric level before the ECG response should be diagnosed abnormal. In healthy persons there is frequently an elevation of the RST which may drop to the isoelectric level after exercise. These workers furthermore did not adhere to the technique we have described in our original reports.<sup>8,15</sup> Instead of a definite number of climbs as obtained from the table, performed in a definite time, *i. e.*, in 1½ minutes, these investigators permitted their patients suffering from angina pectoris to choose their own pace and to continue as long as they could walk until pain set in.

Similarly, Laurentius and Klopffleisch,<sup>5</sup> in the German army, take the ECG after bending the knees 25 times. Here again the amount of work is not standardized and knee-bending is an unaccustomed type of activity for a patient with heart disease. It has been pointed out that an atmosphere of ordinary quiet must be maintained during the test. Excitement and apprehension should be avoided. An ordinary conversational tone and attitude are desired. Too much quiet makes the examinee nervous. We have definitely seen intense nervousness in a patient produce changes that were not present in the "electrocardiogram after the two-step" or on breathing 10% oxygen when the patient was at ease and this has been reported in the literature.<sup>7</sup>

The results of the "electrocardiogram after the two-step" correlate with clinical findings and follow-up.<sup>9,11,12,13,14</sup> We have already given illustrations but there are many others, just as dramatic, which were not included, because in this report we presented only cases in which the patient took the 10% oxygen test as well as the "electrocardiogram

after the two-step." We have had numerous other examples that prove that positive changes in the "electrocardiogram after the two-step" indicate coronary insufficiency and may presage a serious attack of coronary heart disease. For example, a 63 year old officer was examined for some minor complaints. Examination was essentially negative but because of a positive "electrocardiogram after the two-step" the diagnosis of coronary insufficiency was made. Three months later he was admitted to the hospital because of shortness of breath and easy fatigability. The sedimentation rate was increased and the ECG at rest disclosed evidence of myocardial infarction. Another officer was thoroughly examined because he complained of precordial pain. All results were negative including the control ECG at rest. However, the "electrocardiogram after the two-step" was positive, *i. e.*, T1 became inverted, hence a diagnosis of coronary heart disease with coronary insufficiency was made. The basal metabolism was low and thyroid therapy was instituted. Because of its complete success in entirely alleviating the officer's complaint, the diagnosis of hypothyroidism was made and a doubt arose as to the presence of any coronary heart disease. Three months later, however, the patient sustained a severe attack of chest pain for which he was hospitalized for many weeks. Whereas his resting control ECG had always been normal it now presented deeply inverted T waves. The temperature was elevated, the blood count showed a moderate leukocytosis and the sedimentation rate was rapid. In other words, myocardial infarction on the basis of coronary heart disease was the cause of the patient's disability. Altogether, then, a long clinical experience has proven the value of the test for demonstrating diminished function of the heart and coronary insufficiency. This test meets the requirements expressed by Paul D. White,<sup>19</sup> "A simple test is less apt to strain unaccustomed muscles of the subject, less apt to exhaust prematurely a person not in good physical training and more convenient and practical to execute. In fact, such simple exertion as enters into the routine daily life of the patient is best of all."

There is a military application of the "electrocardiogram after the two-step" test. The question of the presence of heart disease or evaluation of cardiac function arises frequently since thousands of men are examined daily. Many officers in the armed forces are 40 or over, an age in which coronary insufficiency is common.<sup>5</sup> Military personnel work under extreme mental and physical strain and are often exposed to a decrease in oxygen supply, so these tests are of particular value. Athletes,<sup>4</sup> soldiers,<sup>18</sup> and fliers<sup>1</sup> may appear in the pink of condition and yet die suddenly under strain. In these men myocardial infarction is found at autopsy as a result of coronary insufficiency. It is therefore suggested that the test be applied to all applicants for the Navy during yearly examination, examinations for promotion, examinations for recall to active service, etc.

**Summary.** An experience gained over many years has proven the practical value of the "electrocardiogram following the two-step exercise" in 3 ways: *First*, the blood pressure and pulse response



indicate circulatory fitness by a standardized measurement of vaso-motor response. *Second*, the ECG changes are an indication of the oxygen supply of the heart muscle itself. *Third*, the control ECG reveals the presence of arrhythmias and is an indication of the condition of the myocardium with the patient at rest.

The test is of importance in differentiating functional from organic heart disease, particularly when physical examination, Roentgen ray film, fluoroscopy and ECG are negative.

Positive changes in the ECG after the "two-step" exercise indicate anoxemia of the heart muscle or coronary insufficiency. Both this test and the 10% oxygen anoxemia test were performed on every person considered in this report. The ECG changes corresponded almost exactly in both tests.

The exercise must be standardized for age and weight since changes occur in normal people if the effort is excessive. Tables giving the number of tips to be performed by normals have been published. In normal persons the blood pressure and pulse return to within 10 points of resting levels in 1½ minutes. The following changes in the "electrocardiogram after the two-step" are considered abnormal: A depression of the RST segment of more than 0.5 mm. in any lead, a change from an upright T wave to an isoelectric (flat) or inverted T wave or T-wave changes in the opposite direction.

In patients with coronary heart disease the test is of particular value in detecting coronary insufficiency when it is latent. In valvular heart disease the test discloses the state of cardiac function and whether the cardiac output is adequate for the coronary arteries.

In patients with hypertension the control ECG often shows evidence of coronary insufficiency and therefore may not change after exercise. There is a lag in return of the blood pressure and pulse following the "two-step" exercise in effort syndrome (neurocirculatory asthenia) and the ECG gives evidence of anoxemia of the heart muscle following exercise. In this syndrome we believe there is a congenitally small, hypoplastic heart which is inadequate on effort. In chest deformities and in congenital heart disease the "electrocardiogram after the two-step" is valuable.

An upper respiratory infection, lung disease, gastro-enteritis, fatigue and lack of sleep may produce abnormal results. The "electrocardiogram after the two-step" is a short, harmless and practical test. It is suggested that it should be a routine procedure in men over 40 in the military service and also for eliminating the unfit for special services where unusual physical and mental strain are experienced, as in aviation, submarine, raider forces, and so on.

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## MORPHOLOGIC OBLITERATION OF CHRONIC MYELOID LEUKEMIA BY ACTIVE TUBERCULOSIS

### REPORT OF A CASE

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A PATIENT with chronic myeloid leukemia in whom the course and pathologic findings were greatly altered by fatal tuberculosis occurring after the diagnosis of leukemia had been established, has been reported by Ulrich and Parks.<sup>5</sup> Autopsy of their patient revealed very little morphologic evidence of leukemia in the organs, although there had been no doubt of the diagnosis during life. Since there is still considerable disagreement concerning the effect of tuberculosis in particular and infection in general on the course of chronic leukemia, the following case is reported. This case is similar to that of Ulrich and Parks, but may be of greater interest because the leukemia was observed for a longer period of time and the time of onset of the active tuberculosis could be dated exactly.

**Case Study.** J. S., a 36 year old white male, entered the Lakeside Hospital in November, 1935, with a diagnosis of chronic myeloid leukemia. The diagnosis had been established at another hospital in January, 1935. At that time the spleen was enlarged and the white blood count was 385,000. He was given Roentgen ray therapy to the spleen and a total thyroidectomy was done because of an elevated basal metabolic rate. Following discharge from the hospital he felt well and was symptom-free when he entered the Lakeside

Hospital for study. The past history and family history were not important. Fowler's solution had been given intermittently before entry to the hospital. The physical examination was entirely negative except for slight enlargement of the liver and spleen, both of which were just palpable at the costal margins on deep inspiration. The temperature was 37.2° C. The red blood count was 4,500,000 per c.mm., with 91% (14.2 gm.) hemoglobin, and the white blood count was 11,500 per c.mm., with 66% polymorphonuclears, 2% myelocytes, 1% eosinophils, 24% basophils, 2% lymphocytes, and 2% monocytes. The blood platelets were abundant. The Kline exclusion test, stool examination, and urine examination were negative. The blood urea nitrogen was 8.3 mg. per 100 cc., blood uric acid 2.9 mg. per 100 cc., and blood cholesterol 162 mg. per 100 cc. During the hospital stay of 6 days, he was asymptomatic. He received 9 to 15 drops of Fowler's solution daily and this medication was continued after discharge.

During the next 4½ years, from November, 1935, until his death in March, 1940, he was admitted to the Lakeside Hospital on 8 occasions and in the interim was followed in the Out-Patient Department. During this time the white blood count varied from 6000 to 170,000 per c.mm., the hemoglobin from 60% (9.4 gm. per 100 cc.) to 90% (14.4 gm. per 100 cc.), myelocytes from 0 to 54%, and myeloblasts from 0 to 10%. The adult polymorphonuclear neutrophils was the predominant cell throughout except for one period when the basophils rose to high levels. Normoblasts were present irregularly in small numbers and an occasional cell undergoing mitosis was found in the peripheral blood.

During the entire 4½ years, he was treated intermittently with Fowler's solution in doses of 9 to 24 minims daily. On 3 occasions Roentgen ray therapy was given when early symptoms of arsenic poisoning made the withdrawal of Fowler's solution necessary. In every instance, there was a good response to both arsenic and Roentgen ray with decrease in the total number of white blood cells, myelocytes, and myeloblasts and increase in hemoglobin. The platelets remained abundant throughout.

Five months before his death, his disease was complicated by the appearance of pleurisy. Immediately following what appeared to be an ordinary head cold, he developed severe pleuritic pain in the left chest accompanied by an audible friction and followed by clinical and Roentgen ray findings of pleural effusion. His temperature became elevated and from then until death ranged between 38° and 40° C. Frequent Roentgen ray examinations showed increase in the pleural effusion and subsequent parenchymal involvement of the left lung field. It was considered possible that the entire pulmonary picture might be the result of leukemic infiltration, but the opinion of most observers that tuberculous was responsible was confirmed when fluid from the left chest was injected into guinea pigs and produced tuberculosis in those animals. Repeated examinations of pleural exudate, sputum, and gastric washings failed to reveal the presence of acid-fast organisms.

During the final 4 weeks of his illness, the white blood count fell to normal and then to subnormal levels. On 1 occasion, it was as low as 2000 per c.mm. The percentage of immature cells decreased, the myelocytes varying from 1 to 10%, and myeloblasts from 0 to 2%. During this final hospital admission, he received Fowler's solution in doses which were generally smaller than he had been receiving before. The average dose during this time was 9 to 12 minims daily. Only for a few days were larger doses given and on 2 occasions, for periods of 1 month each, the drug was discontinued entirely without rapid rise in the white blood count or other evidence of relapse. No Roentgen ray therapy had been given for 8 months before death.

During the 4½ years that he was observed, the liver and spleen had gradually increased in size. Just before death the costal margin, the spleen palpable 10 cm. below the costal margin, and signs of left pleural effusion. On the day of death, the white blood count was 12,850 per c.mm. The differential blood

count revealed 82% polymorphonuclear neutrophils, 9% myelocytes, 2% myeloblasts, 5% basophils, 1% lymphocytes, and 1% monocytes. The hemoglobin was 70% (10.9 gm. per 100 cc.) and the blood platelets were abundant. An autopsy was performed at the Institute of Pathology, Western Reserve University, by Dr. H. K. Giffen, and the principal pathologic diagnoses were as follows: Miliary tuberculosis of the lungs, liver, spleen, kidneys, bone marrow, lymph nodes, and adrenal glands: active, progressive, primary tuberculosis, upper lobe of left lung and hilar lymph nodes: myeloid metaplasia of bone marrow: splenomegaly (910 gm.): hepatomegaly (2470 gm.): chronic adhesive fibrous pleurisy, left: hydrothorax, right (2500 cc.): ascites (2300 cc.): Culture of heart's blood: no growth.

Extensive tuberculosis was found in the upper lobe of the left lung. Elsewhere in the lungs and in the other organs listed above, scattered miliary tubercles were found. Bone marrow sections from sternum, rib, humerus, vertebra, femur, and tibia showed only myeloid elements without marked increase in immature forms and all the marrow contained adequate numbers of red blood elements and megakaryocytes. Leukemic infiltration in other organs was completely absent, even in the spleen which weighed 910 gm.

**Comment.** The tendency for the blood picture to revert towards normal coincident with the progress of the tuberculosis was an interesting feature of this case. Because of the fact that Fowler's solution was administered intermittently, it is impossible to say whether this change was due entirely to the drug or to the infection. It was felt, however, that the smaller doses of the drug would not be expected to produce such a profound effect on the blood and that the infection probably played an important part.

The relationship between leukemia and tuberculosis has been a subject for discussion over a period of 50 years. In various summaries,<sup>1-4</sup> and in numerous case reports the following hypotheses have been advanced to explain the varying clinical and pathologic pictures:

- A. Preexisting leukemia is modified by superimposed active tuberculosis.
- B. Primary active tuberculosis produces a leukemoid blood picture.
- C. The 2 diseases may coexist without much effect on the clinical or pathologic features of either one.
- The individual cases reported in the literature varied widely in their outstanding clinical and pathologic features. However, they may be classified roughly into the following groups:
1. Apparent leukemia observed for a considerable period of time in patients subsequently developing tuberculosis: (a) without change in the expected clinical or pathologic signs of leukemia; (b) with modification towards normal of the expected clinical or pathologic signs of leukemia; (c) with complete or almost complete disappearance of all clinical and pathologic signs of leukemia.
2. Coexistence of leukemia or leukemoid blood picture with active tuberculosis at the time the patient is first observed with development of a, b, or c as under No. 1 above.

The case reported here belongs in Group 1 c. The patient was followed continuously for 4½ years before active tuberculosis developed. During that time there was no doubt about the diagnosis of

chronic myeloid leukemia and not until the tuberculosis became advanced was there any significant change in the signs of leukemia.

It may be significant that we are unable to find any cases in the literature of active chronic tuberculosis observed over a considerable period of time which subsequently developed clinical or pathologic evidence of true leukemia. Those authors who defend the hypothesis that primary active tuberculosis may produce a leukemoid blood picture were unable to give unequivocal evidence of active tuberculosis antedating changes observed in the blood. They inferred from the patient's history that such was the case but positive evidence was lacking. They were never able to state definitely that leukemia had not antedated the tuberculosis.

**Summary.** The case reported here is presented as further evidence that tuberculosis may have a profound influence upon the clinical and pathologic features of chronic myeloid leukemia. It is probable that some factor, produced by infection with tuberculosis, altered the course of the leukemia and caused the reticulo-endothelial system to revert towards normal. In such cases, one is unable to find definite evidence of leukemia at autopsy, although the diagnosis has been established beyond doubt during life. If the mechanism of this process were known, the pathogenesis of leukemia might be more readily discernible.

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## ALLERGIC AGRANULOCYTOSIS WITH COMPLICATIONS

### CASE REPORT

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"There is an old adage in medicine, 'Never despair of the young.' This case is presented because it reiterates this axiom.

The fact that this patient survived these 8 conditions, namely, acute catarrhal fever, lobar pneumonia, agranulocytic angina, acute myocarditis, acute otitis media, bilateral suppurating cervical adenitis and pyelonephritis, concurrently and intercurrently, any of which could have proved fatal, makes the case somewhat of a medical menagerie.

The complications and sequelae and progress of the case also are of interest, because it emphasizes some of the complications which may arise when infection is present with the function of the hematopoietic systems disturbed.

**Report of Case.** This 18 year old white male was admitted to the hospital on 2/28/43, with a diagnosis of acute catarrhal fever. His chief complaints were sore throat and a head cold, duration of 4 days.

The *past history* was irrelevant, except for a tonsillectomy at 7 years of age and a history of hives in the summer, the usual childhood diseases, and pneumonia at 7 years.

The *family history* shows an allergic background (mother suffered from hay fever, sick headaches, colitis and chronic sinus trouble).

Physical examination revealed a well developed and nourished white male 68 inches tall, weighing 149 pounds, apparently not acutely ill. His temperature was 103° F., pulse 135, respiration 35. General physical examination was negative except for a slightly stuffy wet nose, injected throat and posterior pharynx.

He was placed on symptomatic treatment, acetylsalicylic acid 10 grains being the only drug taken internally. Since his temperature receded to normal and he felt well in 2 days, the acetylsalicylic acid was then discontinued. On 3/3/43, the patient's temperature rose to 103° F., accompanied by pain in the right thorax and a productive cough. Roentgen ray and physical findings revealed a lobar pneumonia of the lower portion of the upper right lobe. He was placed on sulfadiazine with 2 gm. for the initial dose and 1 gm. every 4 hours. The red blood count was within normal limits. The white blood count showed 20,000 white cells with an increase in polymorphonuclear leukocytes. The temperature was fluctuant but gradually receded until 3/12/43, when it dropped to normal. The findings in the chest were somewhat diminished but still revealed an unresolved pneumonia of the upper right lobe, which was confirmed by Roentgen ray. The dosage of sulfadiazine was reduced to ½ gm. every 4 hours on 3/13/43. He continued to have a temperature which ranged from normal to 99.6° F. with coarse rales in the upper right thorax and at the base.

On 3/22/43, 19 days after the beginning of sulfadiazine therapy, his temperature became normal and the drug was discontinued after a total consumption of 27 gm. Two days later his blood count showed 5,130,000 R.B.C. and 4550 W.B.C.

Convalescence was uneventful until 3/26/43, at which time he developed a sore throat, slightly tender cervical lymph nodes and pain in the right thorax, productive cough, and rales in the right thorax at the base. Sulfadiazine was again started. On 3/29/43, the cervical lymph nodes became more swollen and tender, and the throat symptoms became worse. Sulfadiazine was discontinued after consumption of 4 gm. and granulocytopenia was suspected. The white blood count was 1950, 97% lymphocytes, and 3% monocytes, showing a complete agranulocytosis. His temperature was 104° F. The diagnosis was changed to malignant granulocytopenia. Small frequent transfusions of 300 cc. of citrated blood daily and 20 cc. of pentnucleotide intramuscularly twice daily was instituted. On 4/1/43, his temperature was 99° F. in the morning but rose to 104° F. at 3 p.m. He became irrational, the cervical lymph nodes became more swollen, the throat became edematous with white ulcerations present on the tonsillar pillars and soft palate. On 4/2/43, pentnucleotide was increased to 40 cc. intramuscularly twice daily and the patient was given 500 cc. of citrated blood intravenously. Appearing to be falling gradually, his temperature being 100.6° F., pulse 130 and thready, he was placed on digitals and put in an oxygen tent.

On 4/3/43, his course was febrile, the blood count showing 2150 W.B.C., with a slight return of immature granulocytes. Citrated blood (300 cc.) was given intravenously. One-half hour later he had a severe chill and his temperature rose to 107° F. A white blood count was taken ½ hour later and revealed 8700 cells. The chest showed impaired resonance, at both bases of the lungs with considerable bubbling rales present. He was expectorating blood-tinged pus which showed numerous short chain streptococci. He complained of pain in the left chest.

On 4/4/43, his temperature was receding, he appeared much improved and

was orientated in all spheres. The Roentgen ray showed unresolved pneumonia in the right upper lobe; right cardiac decompensation, and the ECG showed evidence of widespread myocardial damage. The cervical lymph nodes swelling was less pronounced and less tender; throat ulcers were better, temperature was 100° F; moist rales were present in both bases, more marked on the right, the heart rate was rapid and respiration was increased. Fluids were restricted to 2000 cc. daily, intake and output recorded, and 1 cc. of concentrated liver extract was given in conjunction with the above therapy.

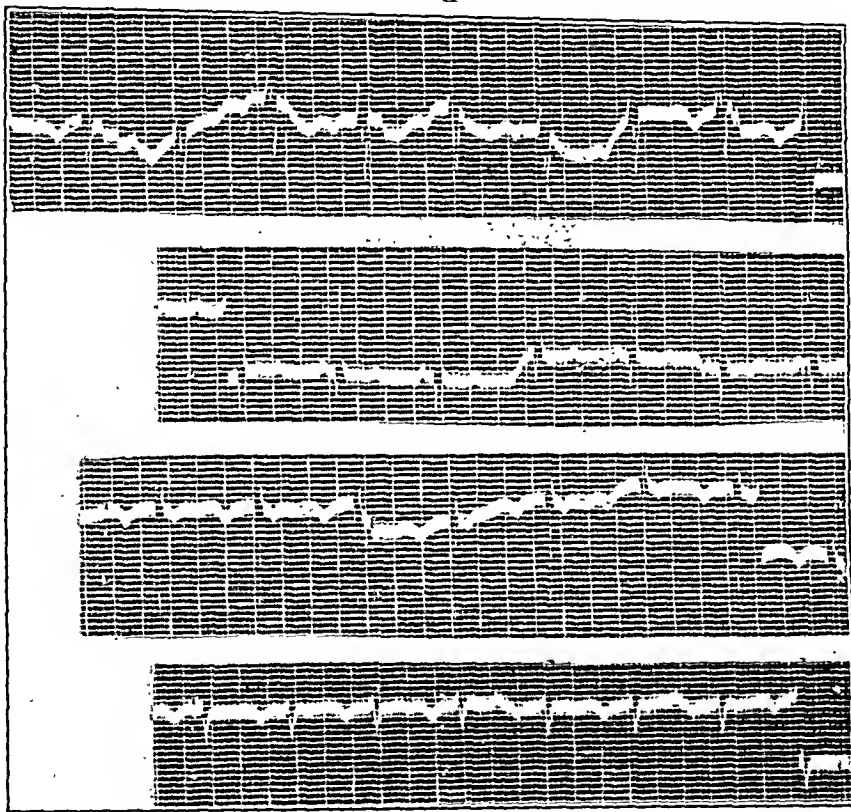


Fig. 1

He appeared much improved on 4/5/43 (Fig. 1), and was taking nourishment. The W.B.C. was 24,000, segments 43%, bands 26%, juveniles 1%, myelocytes 1%, monocytes 5%, lymphocytes 23%, and the R.B.C. was 4,150,000. An acute otitis media developed on the right side and began discharging pus from the ear on 4/8/43. The cervical lymph nodes became woody on palpation, the heart rate and rhythm and tone became normal and pentecloevide therapy was discontinued on this date. His general condition and blood count gradually improved but the angina persisted, and the temperature became elevated. On 4/15/43 Roentgen ray therapy was instituted, 100 R being applied to both sides of the neck. On 4/16/43, the patient was unable to swallow, fluids returning through the nose when he attempted to swallow. The left side of the neck became fluctuant and a large amount of thick yellowish non-odorous pus was evacuated surgically. The W.B.C. rose to 51,000 with a polymorphonuclear leukocytosis predominating. When the right side of the neck was surgically drained on 4/20/43, the temperature and elevated blood count receded and the otitis media cleared up. The blood and lungs were normal on 4/23/43, with the exception of a few coarse rales in the right

base. These findings were confirmed by Roentgen ray examination, but the ECG showed the myocardial damage to persist (Fig. 2). On 4/28/43, there was a sharp rise in temperature and a septic course developed accompanied by pain in the abdomen and flanks. The urine for the first time showed albumin and W.B.C. in increased numbers. Roentgen films of spine, psoas muscles, heart and lungs were negative. The urinalysis continued to show from 40 to 100 mg. per 100 cc. albumin, 15 to 40 W.B.C. per high-power field, and many erythrocytes and granular and hyaline casts. Diagnosis: Acute pyelonephritis. The W.B.C. has ranged from 8000 to 15,000 with a high lymphocyte count predominating.

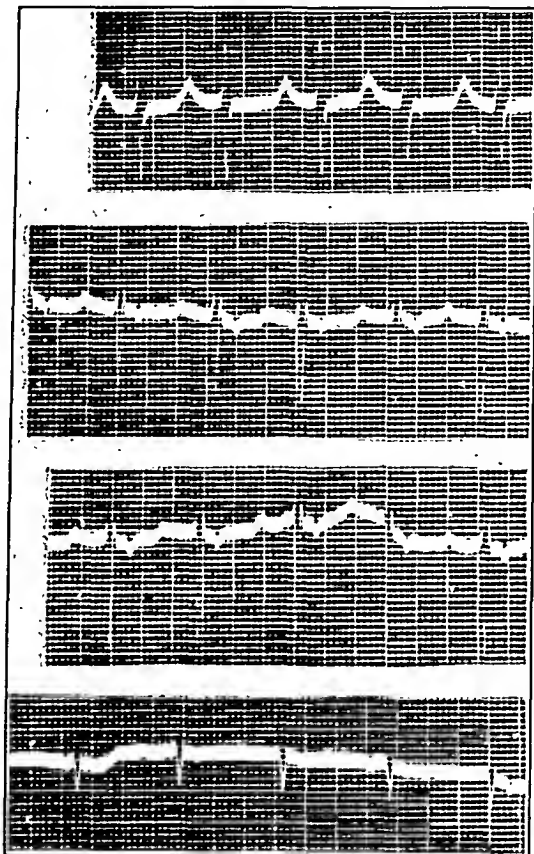


Fig. 2

The urinary findings and elevated temperature have gradually receded, by a watchful, waiting therapy consisting of fluids restricted to 2500 cc. daily, low protein, high carbohydrate diet and recorded intake and output. No drugs were given for fear of inciting the agranulocytosis again. Since 5/9/43, the patient's temperature has remained normal and the urinary findings have returned to normal. The ECG of 5/24/43 (Fig. 3), reveals marked myocardial damage still present but shows improvement in its function. Subsequent tracings may show a greater return to normal than has been seen so far, as his myocardial damage is still recent. These tracings show a prolonged parenchymatous change of a degenerative nature and are not consistent with cardiac damage in pneumonia and acute infections.



The A-V node is not involved and there is a possibility of his other infections being accompanied by a latent rheumatic fever. The blood picture has returned gradually to normal.\*

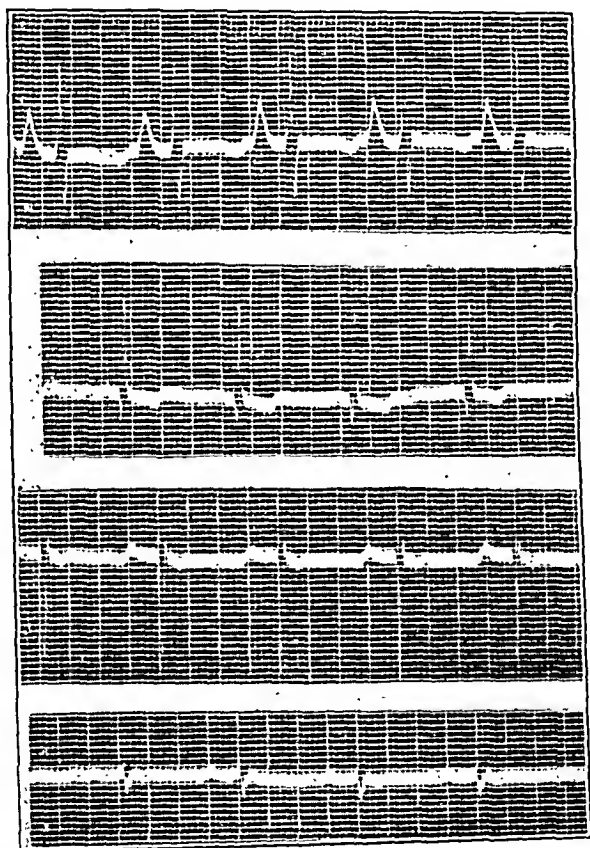


Fig. 3

**Allergy as a Factor in Drug Toxicity and Granulocytopenia.** When hypersensitive tissues are analogous to a loaded gun or an electrical current. When the inciting allergens, which may be compared to the trigger or switch are encountered, this state of equilibrium may become disrupted and any of the shock tissues may become involved.

Thus, in our present day of synthetics and new drugs, allergy has assumed a larger field than the hay fever, urticaria and asthma of the past. "A person who is allergic to ordinary innocuous agents is apt to be more sensitive than normal to many or all toxic agents, which is well illustrated in agranulocytic angina. Patients with this condition are almost universally allergic to many substances."<sup>10</sup>

Rowe<sup>15</sup> is of the opinion that agranulocytic angina is due to allergy in the blood forming cells of the bone marrow.

Beckman<sup>2</sup> states that granulocytopenia occurs more frequently in children in the first 10 days of therapy and that true agranulocytosis

\* Since submitting this paper for publication, another ECG was made on the patient, which revealed a normal tracing (Fig. 4).

with angina and the typical blood picture is rare, but such are being reported. Holten,<sup>11</sup> borrowing Quirk's hypothesis, infers that it is an Arthus phenomenon localized to the leukopoietic part of the bone marrow. Curry,<sup>5</sup> Levin and Bethell<sup>14</sup> have reported cases of agranulocytosis due to sulfadiazine, the latter reporting a fatal case. Conti<sup>3</sup> reported a fatal case due to sulfapyridine.

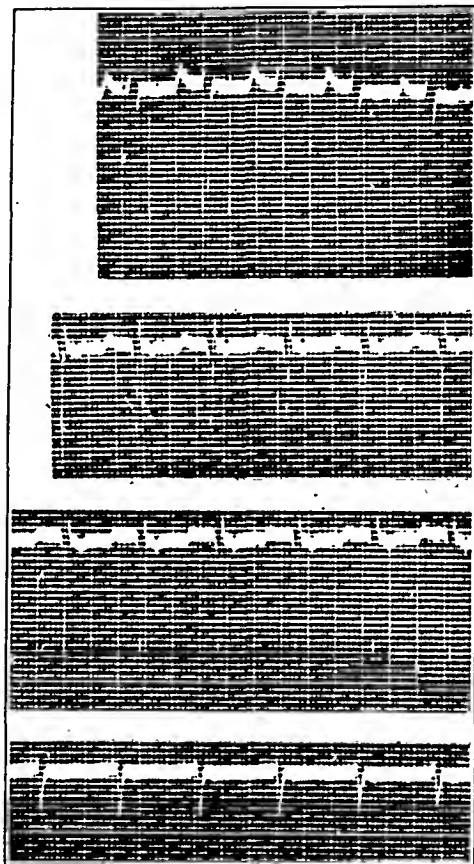
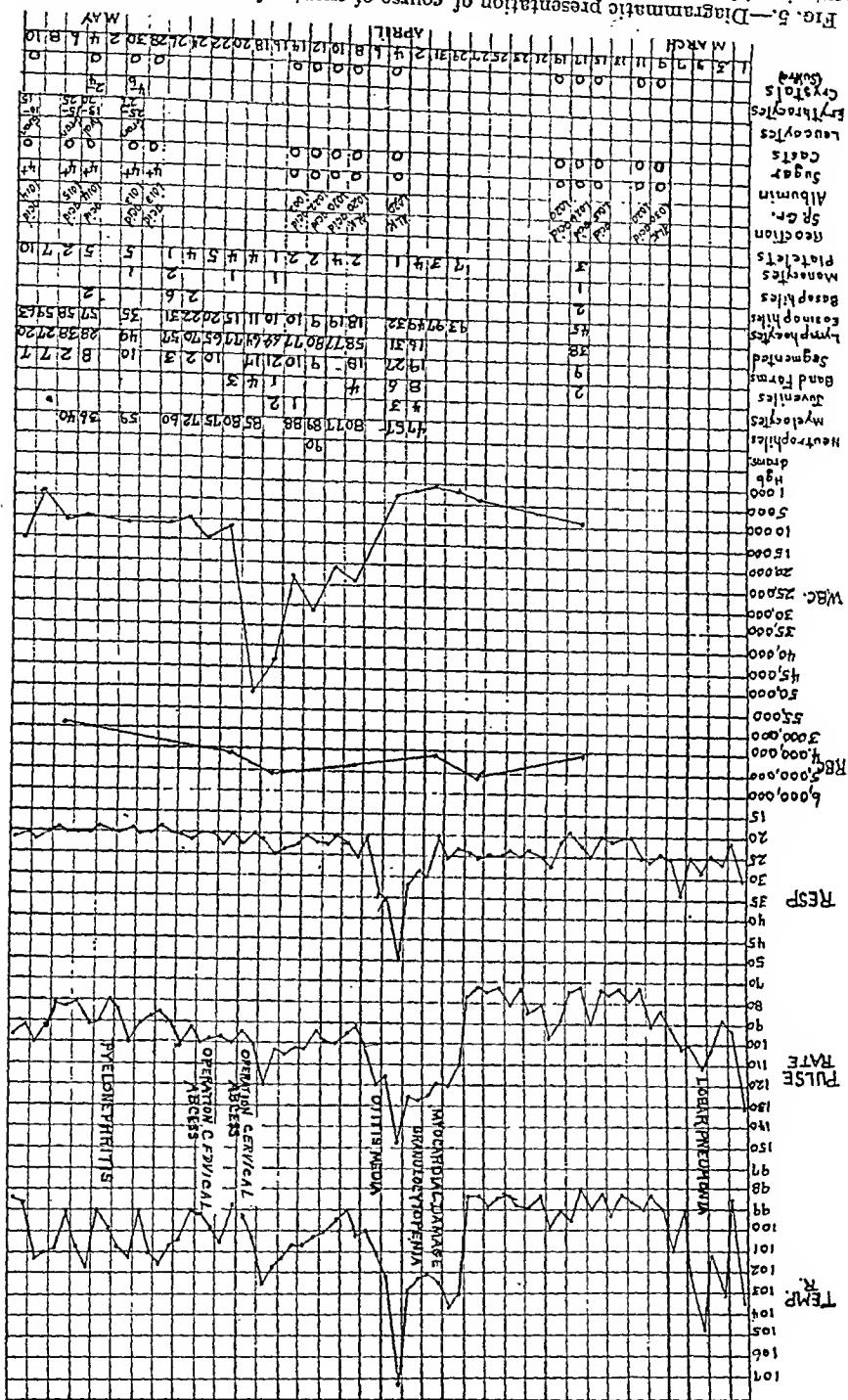


Fig. 4

Rinland, Strauss and Peterson<sup>8</sup> reported a leukopenia incidence of 1.67% in a series of 776 cases who were taking sulfadiazine. Keefer<sup>12</sup> reported an incidence of 2% in his series of cases. Rippin<sup>9</sup> states that depression of the bone marrow may occur at any time during the administration of sulfonamides but that no cases have developed within 12 days of such therapy. Dameshek and Wolfson<sup>6</sup> believe that sulfathiazole is of value in combating the secondary sepsis in agranulocytosis and report 2 cases to substantiate their belief. Authorities on the subject, however, are not in accord with this idea and it is generally recognized that sulfonamides produce granulocytopenia and agranulocytosis by their toxic and allergic effect on the blood forming elements.

Sutliff *et al.*<sup>18</sup> found 1 fatal case of sulfonamide toxicity in every 2571 deaths from all causes, with a frequency of 1 sulfonamide death



among 161 reported pneumonia deaths. He estimated that there was 1 sulfonamide death for every 1610 pneumonia cases in which a sulfa drug was employed.

Perrin H. Long<sup>12</sup> points out that 10 to 15 million people received one of the sulfonamide derivatives in 1941 and warns that there is a possibility that a large per cent of our population may become sensitized to these drugs, because the frequency with which such sensitivity arises is apparent. He cites that the sulfonamide derivatives produced complications as follows:

Sulfanilamide 11.9%; sulapyridine 15.9%; sulfathiazole 18.6%; sulfadiazine 6.5%.

**Discussion.** The fact that this patient had an allergic background is substantiated by his past history of recurrent urticaria and by his family history. It is significant because it emphasizes the fact that he was born with a tendency to become sensitive.

In most instances where a patient develops the classical picture of agranulocytic angina, they are sensitive to other allergens or noxious substances.

Patch and intradermal tests with sulfadiazine were done, but all were negative. This was to be expected. According to Sulzberger<sup>13</sup> skin tests are of little value in drug toxicity due to the fact that the drugs are primarily urticarogenic and one may be clinically sensitive to a drug while showing negative skin tests. Desensitization by the use of minute doses of the drug was not attempted, neither were any sulfonamide derivatives or coal tar or arsenical preparations allowed this patient because his condition was too grave to run the risk of inciting his sensitiveness again. However, Flippin (9) states that leukopenia or neutrophilia *per se* do not contraindicate sulfadiazine therapy and that these conditions will usually disappear as the infection is brought under control. He administers sulfonamides in cases with a history of previous sulfonamide toxicity and follows the patient closely. The fact that the drug was administered over a period of 19 days, discontinued and then resumed may have been a factor in the allergic response. Most authorities state that toxic reactions generally occur after the 12th day and granulocytopenia occurs between the 17th and 25th day of administration of the sulfa drug.

The reaction of the blood transfusion may have played a part in stimulating the bone marrow to resume production of granulocytes. Cross<sup>1</sup> reported a case which recovered from agranulocytosis due to sulapyridine after a rigor during a transfusion.

Although this patient ran the gamut of the medical armamentarium for granulocytopenia, one cannot say that any one treatment was responsible for his recovery. It would be safer to say that it was more than likely due to the vigor of a youthful body which adjusted itself to its environment with the help of a fighting spirit.

**Summary and Conclusions.** A case of allergic agranulocytosis with probable etiologic agent and the bone marrow serving as the trigger point of sensitivity, or shock tissue.

Charts, ECG tracings and Roentgen ray pictures of the case emphasize the various complications and sequelae and progress of the case. This case illustrates the grave complications which may arise from the sulfa therapy and is a warning to overdosing and prolonged use of a toxic drug. Indiscriminate prescribing of these potentially toxic drugs should be guarded against in minor conditions especially to a person who has an allergic background. This case also illustrates some of the many complications which may occur when the function of the hemopoietic system is disturbed.

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## BLOOD DIASTASE VALUES IN MUMPS AND MUMPS PANCREATITIS

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PANCREATITIS has been shown<sup>1,2</sup> to be accompanied by a prompt rise in blood diastase, and the estimation of this enzyme in the blood is used for diagnosis of pancreatitis in differentiation from acute surgical syndromes.<sup>3</sup> A survey of the literature has revealed no published observations of diastase values in the pancreatitis complicating mumps. In the course of an epidemic at Camp Adair, from January to June 1943, the author saw over 600 cases of mumps. Occasional instances of pancreatitis were diagnosed clinically<sup>4</sup> among these cases, and the

diagnosis apparently verified by demonstrating elevated blood diastase values. It was noted that the elevated values would frequently be sustained for days, up to 3 weeks in one case, before dropping to normal, and that these sustained values were accompanied in most cases by symptoms and signs suggestive of continuing pancreatic disease. The symptomatology suggestive of pancreatitis included a rise of temperature, leukocytosis, anorexia, nausea with or without vomiting, epigastric and left upper quadrant distress, severe abdominal distention, deep tenderness over the region of the pancreas, and occasional muscular rigidity of the epigastrium, left upper quadrant and left loin. Pain was vaguely localized to the upper abdomen, was frequently cramping, and was reproduced by deep pressure over the pancreas. Blood diastase values ran as high as 600 mg. per 100 cc. Inasmuch as the salivary glands produce diastase, it seemed advisable to determine whether mumps, uncomplicated by pancreatitis, is accompanied by elevation of the blood diastase. Dunlop<sup>4</sup> found increased urinary diastase values in mumps, but Heifetz, Probststein and Gray<sup>5</sup> were unable to demonstrate a rise in blood diastase in a small series of cases. According to these authors, the only diseases of the salivary glands known to produce elevated blood diastase values are suppurative and ductal obstruction.

**Method.** Routine blood diastase values were taken on admission of a series of cases of mumps, and again prior to discharge. Except in occasional instances in which administrative reasons for discharge prevailed, patients with elevated diastase values were retained until these dropped to normal. During the course of illness, diastase values were also determined whenever symptomatology indicated the possibility of pancreatitis. Hospitalization time was 2 weeks as a minimum, and as much longer as signs or symptoms of any manifestation of mumps persisted. All admission and discharge values are included in this analysis, and none disallowed. Diastase determinations other than those on admission and on discharge are not included in the tabulation.

TABLE 1.—DIASTASE VALUES IN MUMPS, ON ADMISSION AND DISCHARGE, OF A SERIES OF 89 CASES

Diastase values (mg. per 100 cc.)		No. on admission		No. on discharge	
Subnormal, below 60.		3 (3½%)		16 (20%)	
Normal, 60-180		20 (23½%)		57 (71%)	
Above normal.		62 (73%)		7 (9%)	
180-300	.	.	.	5	.
300-400	.	.	.	1	.
400-500	.	.	.	1	.
Over 500	.	.	.	0	.
Total		85 (100%)		80 (100%)	

Discrepancies in the totals are due to failure to obtain admission values in 4 patients, and discharge values in 9 patients, of this series.

The method of diastase determination used by the laboratory was that of Somogyi,<sup>12</sup> and consisted briefly in blood sugar determination before and after incubation of a blood sample with starch solution for 30 minutes. The difference between the two determinations is the diastase value, recorded as milligrams per 100 cc. of reducing substances. Normal values are stated by Somogyi<sup>11</sup> to fall within the limits of 60 and 180 mg. per 100 cc. Four per cent of normals

were found by Somogyi to fall below 60 mg., none above 180 mg. A limited number of determinations on normal controls at this hospital were within the limits of 60 and 180 mg. Somogyi stresses the normal constancy of an individual's diastase level, there being no tendency to physiologic fluctuation.

**Results.** The table lists the diastase values on admission and on discharge of the series of 89 cases of mumps. Three-fourths of the patients on admission were found to have abnormally high diastase levels. Three patients had subnormal levels on admission. On discharge, 20% were subnormal, and only 9% above normal. In this series of 89 cases, there were 13 instances (15%) of pancreatitis, as indicated by symptomatology associated with a rise of the diastase value above the admission level. An examination of the admission diastase values of these 13 cases revealed that all but 1 were above normal, an incidence of 92% as compared with 73% for the series as a whole.

An attempt was made to correlate the degree of abdominal distention and that of pancreatic tenderness, as noted at the time of admission, with the diastase value on admission, but no statistically significant correlation was found. Neither did the 13 cases which subsequently developed pancreatitis exhibit these clinical findings on admission to any greater degree than the series as a whole.

**Discussion.** Diastase is known to be produced by the pancreas and by the salivary glands. In both instances, however, this production is released through excretory ducts and does not normally reach the blood stream. Injury to these glands does not decrease the blood diastase level, having in fact the opposite effect in pancreatitis.<sup>5,8,10</sup> and possibly in salivary adenitis.<sup>4</sup> Hepatic impairment, however, causes a fall in blood diastase<sup>7</sup> and it is postulated, therefore,<sup>3</sup> that the diastase normally found in blood has its origin in the liver.

The number of subnormal diastase levels on recovery from mumps was surprisingly large, and is best accounted for by assuming some hepatic functional impairment.<sup>1</sup> In some cases of pancreatitis also, it was noted that the high diastase values fell to subnormal levels for a few days before returning to normal. Inasmuch as mumps is regarded as a generalized systemic disease,<sup>13</sup> with a predilection for glandular tissues, it would not seem too far-fetched to suppose the existence of hepatic involvement in these cases. This is a possibility which could be investigated by use of additional liver function tests in following the course of mumps.

The high percentage of increased diastase values found on admission must indicate either an unexpected frequency of subclinical pancreatitis or a parenchymatous injury of the salivary glands. It is possible that both possibilities contribute to the rise in diastase. *A priori*, knowing that the salivary glands produce diastase, and that their histologic appearance is similar to that of the pancreas except for the absence of islands of Langerhans, it might be expected that the increased diastase of the blood is secondary to salivary gland injury, in a manner analogous to that which follows pancreatic injury. However, the fact that those cases which subsequently developed pancreatitis showed an even

greater percentage of increased diastase values on admission, would indicate either that these are the more severe cases of mumps, with greater salivary gland involvement, or that subclinical pancreatitis was already present and was accounting in part, at least, for the elevation in the blood diastase.

There appeared two ways in which this problem might be pursued. One was the method of Lagerlöf,<sup>10</sup> who has shown the diagnostic value in acute pancreatitis of diminished diastase content in the duodenal secretion of pancreatic juice following the injection of secretin. Though well established by Lagerlöf's careful and thorough work, this method of determining the presence or absence of pancreatitis was, unfortunately, not used by us because of the necessity of passing duodenal tubes routinely in our mumps patients. Instead, determinations of blood lipase have been shown to be increased in the blood in pancreatitis,<sup>11</sup> but should not be affected in salivary adenitis since the salivary glands do not produce lipase.

After patient effort and careful control studies, however, the laboratory personnel and the author were convinced that quantitative blood lipase studies were not sufficiently reliable—at least in our hands. It is significant that Lagerlöf<sup>10</sup> has arrived at the same conclusion regarding the blood lipase determinations; this does not seem to be the opinion reflected in the American literature, however.

**Summary.** Blood diastase values were determined in a series of 89 cases of mumps on admission to hospital and on discharge. Seventy-three per cent were above normal on admission, and 9% on discharge. Fifteen per cent of this series showed evidence of pancreatitis sometime during the course of illness.

Those cases which later developed pancreatitis had an especially high percentage of increased diastase values on admission. Studies intended to illuminate the possible rôle of the pancreatitis in producing these values did not prove feasible under our circumstances.

This study was under the supervision of Lt.-Col. Frederick Kellogg, Medical Service, Bolker, Laboratory Service.

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## OBSERVATIONS ON THE ORAL ADMINISTRATION OF CITRATED

## BLOOD IN MAN

## III. THE EFFECT ON TEMPERATURE AND THE WHITE BLOOD

## CELL COUNT\*

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Fever is said to occur in 58 to 91% of patients with bleeding peptic ulcer.<sup>1</sup> It was present in 122 (84%) of 145 patients with bleeding peptic ulcer seen at the Cincinnati General Hospital between October 1, 1937 and March 30, 1943, who were free of complications known to be accompanied by fever. Fever was present in 100 (81%) of 123 additional "uncomplicated"† cases of hematemesis and melena due to ruptured esophageal varix, gastric carcinoma, gastritis, or undetermined cause. Pyrexia usually occurs within 24 hours after hematemesis or melena, lasts from a few days to a week or slightly longer, and may reach a maximum of 103° F. In cases of bleeding peptic ulcer it more frequently follows massive or moderately severe than mild hemorrhage.<sup>1,3</sup>

Perusal of the scant literature on this subject indicates that the cause of the fever is not known. According to Dill and Isenhour,<sup>1</sup> numerous factors have been invoked, including absorption of blood decomposition products, reduction in blood volume, anemia, associated gastritis, or increase in lability of the heat-regulating center as a result of asthma or shock. According to these authors, the absorption of blood from the intestinal tract was assigned as the cause of the fever by Eichorst, Leichtenstern, Riegel, and Krehl. Hurst<sup>2</sup> stated in 1929 that "the old view that the pyrexia is due to the absorption of products of putrefaction of blood is generally correct."

As far as we are aware, the first attempt to evaluate experimentally the role of blood in the intestinal tract in the production of the fever was made by Dill and Isenhour.<sup>1</sup> They administered to 4 dogs, by stomach tube, approximately one-fourth the volume of each animal's blood. There was no effect on the temperature curves during the following 10 days. They also found that removing one-fourth of the dog's blood volume did not produce fever, nor did fever result from

\* This study was aided by a grant from Parke, Davis & Company, through the cooperation of Dr. E. A. Sharp.

† Uncomplicated by a disease which could of itself produce fever.

the combined removal and introduction of this blood into the intestinal tract. They introduced into the stomachs of 4 human subjects 500 to 600 cc. of citrated blood obtained by phlebotomy from patients with congestive heart failure and found no significant elevation of temperature during an ensuing 10-day period. They concluded that blood in the intestinal tract of man does not of itself cause a significant rise in temperature.

**Experimental Observations.** We thought it desirable to determine the effects of larger volumes of blood than used by these authors, since we believe that patients with hemorrhage from the upper digestive tract frequently lose a liter or more of blood. Accordingly, 6 patients were selected for study; 1 was 43 years old and the remainder between 53 and 60 years of age. Two had duodenal ulcer (1 with recent hemorrhage); 1, a jejunal ulcer; 2, chronic gastritis, and 1, polycthemia. The patient with the recent hemorrhage had had fever of 1 week's duration following his hemorrhage, with a maximum elevation of 101° F. He had a minimum red cell count of 1,500,000 and a count of 3,300,000 at the time he was given 1000 cc. of citrated blood. The patient with polycthemia received 800 cc. of his own blood removed by phlebotomy 3 hours before. Of the remaining 4 patients, 1 received 750 cc. of his own blood and 2 received 1000 cc.; 1 received 1850 cc. of citrated human blood obtained from the Blood Bank of the Cincinnati General Hospital.

The blood was given by stomach tube. In amounts up to 1000 cc. it was allowed to flow into the fasting stomach by gravity over a period of 30 to 60 minutes. The 1850 cc. of blood was divided into 3 portions given at 4-hour intervals. A hypodermic injection consisting of codeine sulfate gr. 1 and atropine sulfate gr. 1/150 preceded the administration of the blood in order to prevent too rapid passage through the intestinal tract. None of the subjects vomited the blood. The patients with peptic ulcer were on a Melen-gracht or bland diet, while the others were on a regular diet during the period of this study.

Figure 1 is a record of the oral temperatures generally taken at 4-hour intervals for 48 hours preceding the administration of the blood and 72 hours following. A significant rise in temperature did not occur in any of the 6 subjects.

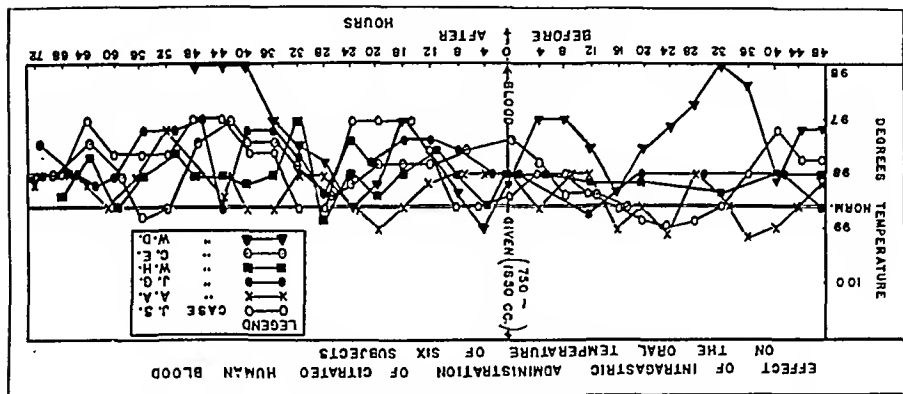


FIG. 1

Contrary to the experience of Dill and Isenhour,<sup>1</sup> leukocytosis has frequently followed hematemesis or melena due to various causes in patients admitted to the Cincinnati General Hospital, particularly in patients seen within 48 hours after the occurrence of hemorrhage. In 95 (65%) of 145 cases of "uncomplicated" bleeding peptic ulcer, the white cell count varied from 10,000 to

25,000, while a leukocytosis of 10,000 to 25,000 was present in 74 (60%) of 123 cases with hematemesis or melena due to ruptured esophageal varix, gastric carcinoma, gastritis, or undetermined cause.

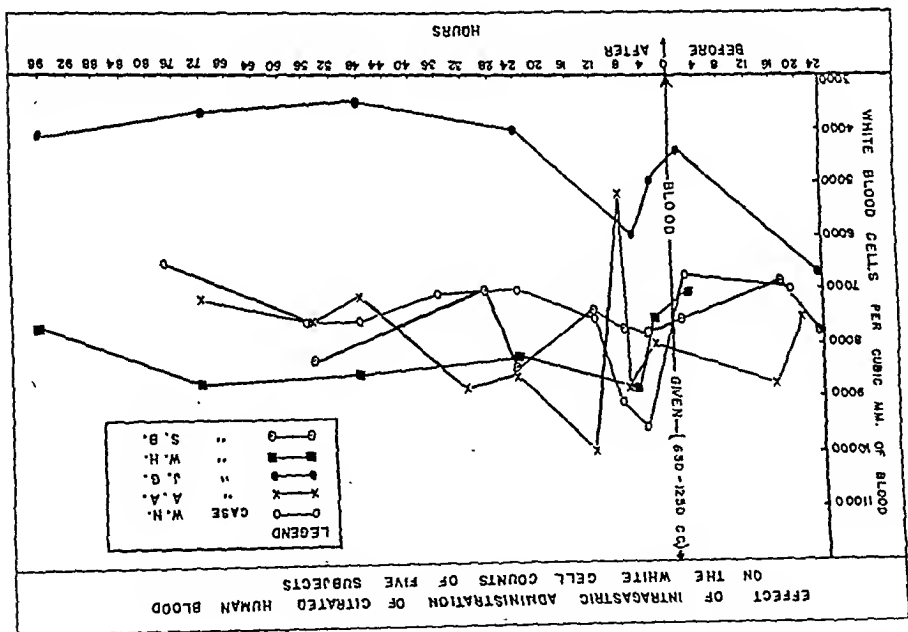


Fig. 2

The mechanism of the leukocytosis is presumably the same as that which follows hemorrhage in general. We were, nevertheless, interested to determine whether the presence of blood in the intestinal tract of itself had any influence on the leukocyte count. Accordingly, white blood cell counts were made at frequent intervals during the day preceding and at frequent intervals during the 72 to 96 hours following the intragastric administration of blood. No significant changes occurred (Fig. 2). Three of the subjects used in this study were the same as those used in the temperature study (2 with duodenal ulcer and 1 with chronic gastritis). The remaining 2 subjects were 33 and 37 years old; 1 was convalescing from pneumococcal pneumonia, while the other had recovered from a gastro-enteritis.

**Summary.** There was no significant elevation of temperature in 6 human male subjects within a 3-day period following the intragastric administration of 750 to 1850 cc. of citrated human blood. There was no significant change in the white blood cell count of 5 human male subjects in a 3- to 4-day period following the intragastric administration of 650 to 1250 cc. of blood.

**Conclusion.** The presence of citrated human blood in the intestinal tract of men does not produce fever or leukocytosis within a period of 3 to 4 days.

We wish to express our thanks to Dr. Paul Hoxworth, Director of the Red Cross Transfusion Service, for his cooperation in this study.

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# THE INFLUENCE OF LARGE DOSES OF VITAMIN A UPON THE PLASMA VITAMIN A LEVEL\*

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Under physiologic conditions and with average nutrition the plasma vitamin A level remains constant during the day and on consecutive days.<sup>15,31,38</sup> Ingestion of high doses of vitamin A raises the plasma vitamin A level temporarily, and after 24 hours the previous level is usually reached.<sup>1,5,32,37,39,42,43</sup> The temporary rise with ensuing drop has been designated as tolerance curve. It has been shown that the maximal increase of the plasma vitamin A level in the tolerance curve is not determined by the vitamin A concentration of the liver but that it depended chiefly upon intestinal absorption.<sup>39</sup> The flat tolerance curves found in liver diseases<sup>5,39,42</sup> were explained by impaired intestinal absorption due to liver damage.

Assuming that the tolerance curve mirrors the intestinal absorption of vitamin A, several problems arise which form the basis of this paper. (1) Is there a relation between the fasting plasma vitamin A level and the maximal response in the tolerance curve? (2) Is there a relation between the lipid content of the blood and the response in the tolerance curve? This question arises because Josephs<sup>19,20,22</sup> demonstrated in animals and children a relation between the response of the serum vitamin A level to the intake of high doses of vitamin A and the blood lipids as carriers of vitamin A. (3) Is there a difference in the response in the tolerance curve to equal amounts of esterified vitamin A or vitamin A alcohol or carotene? (4) Is there a more marked response in the tolerance curve when vitamin A is given orally or intramuscularly? (5) Does the simultaneous administration of vitamin E influence the tolerance curve? This question is raised because vitamin E as an antioxidant may reduce the destruction of vitamin A in the intestinal lumen and thus increase its absorption.<sup>21,48</sup>

**Material and Method.** Hospital patients suffering from various diseases were studied. Each subject received 75,000 I.U. of vitamin A ester in 2 cc. oil mixed in fruit juice. The plasma vitamin A level was determined before, and 3, 6, and 24 hours after administration of vitamin A. The Carr-Price

\* Supported by a grant from the Scientific Committee of the American Medical Association and from the S. M. A. Corporation, Chicago.  
† Distilled vitamin A concentrate (natural ester form, distilled from fish liver and vegetable oil) containing 200,000 U.S.P. XI units per gram, generously supplied by Distillation Products, Inc., Rochester, N. Y.

reaction was used for determination following the method of Kimble.<sup>24</sup> The measurements were made either by the Sheard-Sanford photoelectric colorimeter or by the copper sulphate standard according to the method of Josephs.<sup>19</sup> The details of the procedure were described in a previous publication.<sup>40</sup> In 20 cases, the total plasma fat<sup>45</sup> and in 88 cases the total blood cholesterol<sup>25</sup> were determined. Furthermore, in 7 cases the first tolerance curve obtained after vitamin A esters was compared with a second obtained after ingestion of 75,000 I.U. vitamin A alcohol\* in 2 cc. oil mixed in fruit juice. The second tolerance curve was made a few days after the first. In 11 cases, the tolerance curve was repeated after administration of beta carotene, equivalent to 75,000 I.U. vitamin A in 2 cc. corn oil. In 14 cases, the tolerance curve was compared with the changes of the vitamin A level after the intramuscular administration of 75,000 I.U. vitamin A.<sup>†</sup> In 13 cases, the tolerance curve was repeated with simultaneous administration of 50 mg. alpha-tocopherol.<sup>‡</sup>

**Results.** I. *Relation Between Fasting Plasma Vitamin A Level and the Maximal Increase in the Tolerance Curve.* In Figure 1 the maximal increase in the tolerance curve is plotted against the fasting vitamin A level. There was no relation between the fasting plasma vitamin A level and the maximal increase in the tolerance curve except that with a fasting level of above 50  $\mu$ g., a level in the high ranges of normal, the response in the tolerance curve was always satisfactory. However, patients with fasting levels of up to 40  $\mu$ g. had occasionally no response or almost none. Liver damage (as evidenced by jaundice) exerts an influence on intestinal absorption. Jaundiced patients with zero or nearly zero plasma vitamin A levels had usually no response, but sometimes the maximal increase was quite marked. If the jaundice cases are grouped according to duration of liver damage, it is evident that in patients with acute liver damage, as in acute hepatitis or in secondary hepatitis of obstructive jaundice, no or very slight response was found; whereas in patients with chronic liver damage, as in cirrhosis, sometimes a satisfactory response in the tolerance curve was observed.

II. *Relation Between the Maximal Increase in the Tolerance Curve and the Concentration of Fat and Cholesterol in the Blood.* The blood lipids are usually considered the carriers of vitamin A. The possibility thus arises that the amount of available carrier substances may influence the response in the tolerance curve. However, a comparison between the total blood lipids and the maximal increase of the plasma vitamin A level following the vitamin A administration reveals no relation (Fig. 2.) High maximal increases were found with both high and low blood lipids. The lipids themselves did not rise after administration of vitamin A. The maximal increase of the plasma vitamin A level after administration of vitamin A plotted against the total cholesterol in the blood (Fig. 3) shows no consistent relation. In fact, high cholesterol levels were frequently associated with a lack of response in the tolerance curve. This observation, however, was made mostly in patients with obstructive jaundice due to a malignant condition of the

\* Furnished as vitamin A alcohol concentrate containing 600,000 units.

† Kindly supplied in ampules containing 100,000 U. per cc. by E. R. Squibb & Son, New Brunswick, N. J.

‡ Courtesy of Distillation Products, Inc., Rochester, N. Y.

head of the pancreas or common duct. Similar to the total lipid content, the total cholesterol also failed to rise following the administration of 75,000 I.U. vitamin A.

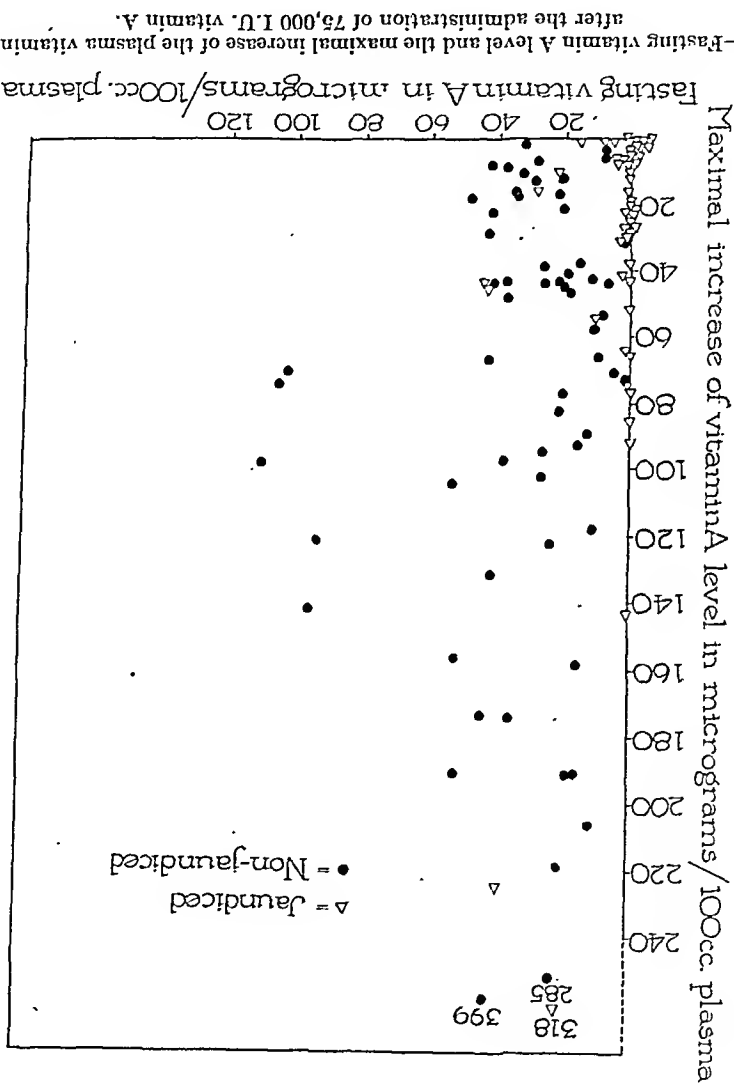


Fig. 1.—Fasting vitamin A level and the maximal increase of the plasma vitamin A level after the administration of 75,000 I.U. vitamin A.

III. Influence of Type of Vitamin A Administered. 1. Tolerance Curves After Administration of Vitamin A alcohol: In 7 patients, 3 without and 4 with jaundice, the vitamin A tolerance curve after ingestion of the usual 75,000 I.U. vitamin A ester was compared with the tolerance curve obtained after administration of 75,000 I.U. vitamin A alcohol. Only a slight difference in the maximal increase in the two tolerance curves was noted.

2. Tolerance Curves After Carotene Administration: In 11 patients (6 with jaundice) the vitamin A tolerance curve after ingestion of 75,000 I.U. vitamin A was compared with the change of the plasma

vitamin A level after ingestion of beta-carotene equivalent to 75,000 I.U. vitamin A. The vitamin A level did not rise appreciably after the carotene intake, although normal vitamin A tolerance curves were obtained in some cases (Fig. 4). The plasma carotenoid level itself was slightly elevated after carotene administration, whereas vitamin A ingestion changed only occasionally the carotenoid level.<sup>6</sup>

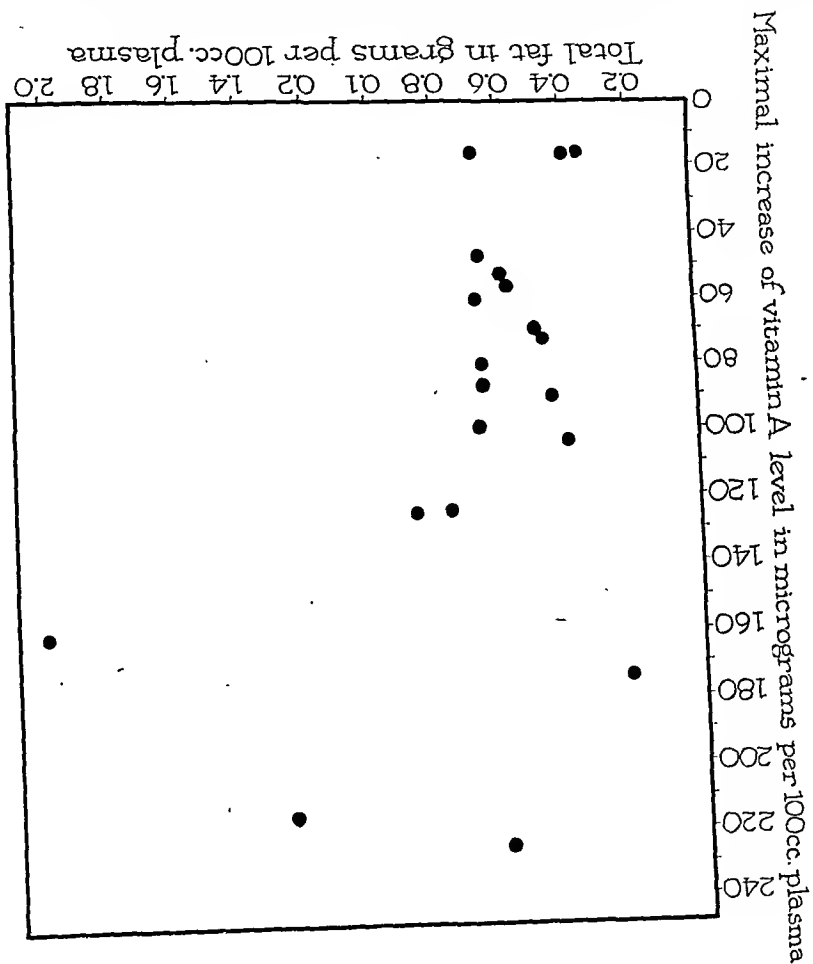


Fig. 2.—Total fat in the plasma and the maximal increase of the plasma vitamin A level after the administration of 75,000 I.U. vitamin A.

IV. *Influence of Route of Administration.* In 14 cases (hospital controls and patients with various diseases, including liver disease) the tolerance curves after the oral intake of 75,000 I.U. of vitamin A were compared with those made after intramuscular injection of 75,000 I.U. vitamin A. No response in the tolerance curve (Table I) after intramuscular injection of vitamin A was noted; the average maximal rise being only 4  $\mu$ g., in contrast to the usual response following the oral intake of the same dose of vitamin A. In only 2 cases was the plasma vitamin A level higher 24 hours after the intramuscular administration

than prior to it; whereas in the other 12 cases, the curve had returned to the original level.

TABLE 1.—AVERAGE PLASMA VITAMIN A LEVEL IN 14 CASES, BEFORE AND AFTER ORAL AND INTRAVASCULAR ADMINISTRATION OF 75,000 I.U. VITAMIN A

Plasma vitamin A level in micrograms per 100 cc.		
Oral administration		Intravascular administration
Before.	37	44
After 3 hours.	138	48
After 6 hours.	200	48
After 24 hours.	62	45

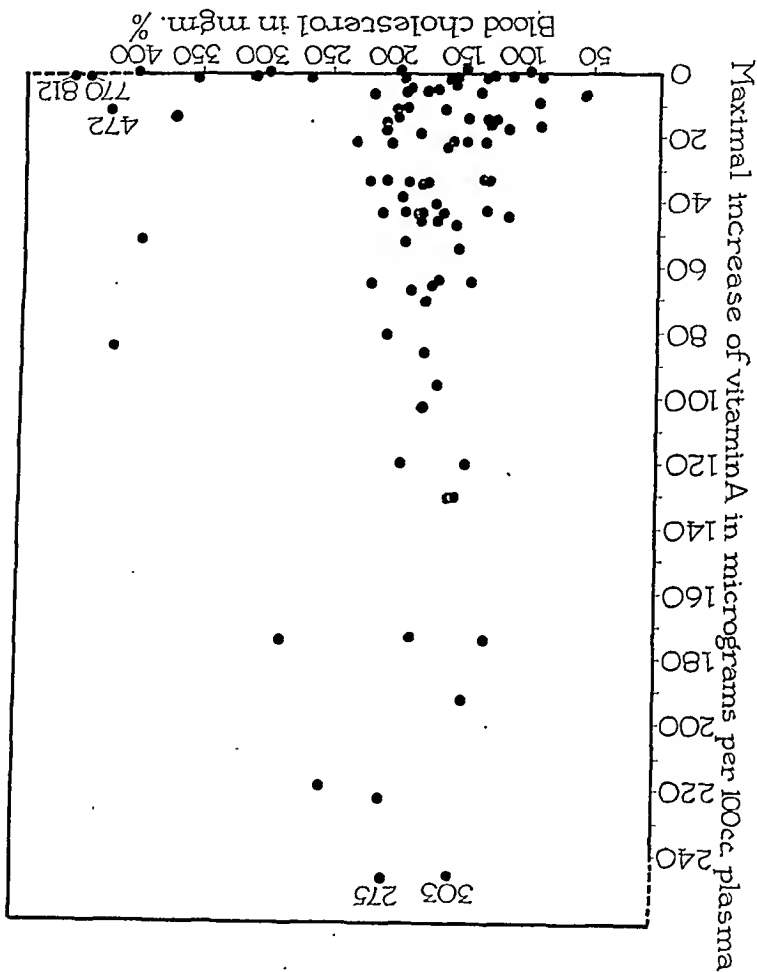


Fig. 3.—Total blood cholesterol and the maximal increase of the plasma vitamin A level after the administration of 75,000 I.U. vitamin A.

V. Influence of Vitamin E on the Tolerance Curve. When the vitamin A tolerance curves were repeated with the simultaneous administration of 50 mg. alpha-tocopherol some variations of the tolerance curve in both directions were noted. The averages of the two tolerance curves, however, were similar (Table 2).



TABLE 2.—AVERAGE PLASMA VITAMIN A LEVEL IN 13 CASES, BEFORE AND AFTER INGESTION OF 75,000 I.U. VITAMIN A WITH AND WITHOUT SIMULTANEOUS ADMINISTRATION OF 50 MG. ALPHA-TOCOPHEROL

Plasma vitamin A level in micrograms per 100 cc.			
Without alpha-tocopherol		With alpha-tocopherol	
Before.	26	33	33
After 3 hours.	68	80	80
After 6 hours.	87	90	90
After 24 hours.	36	39	39

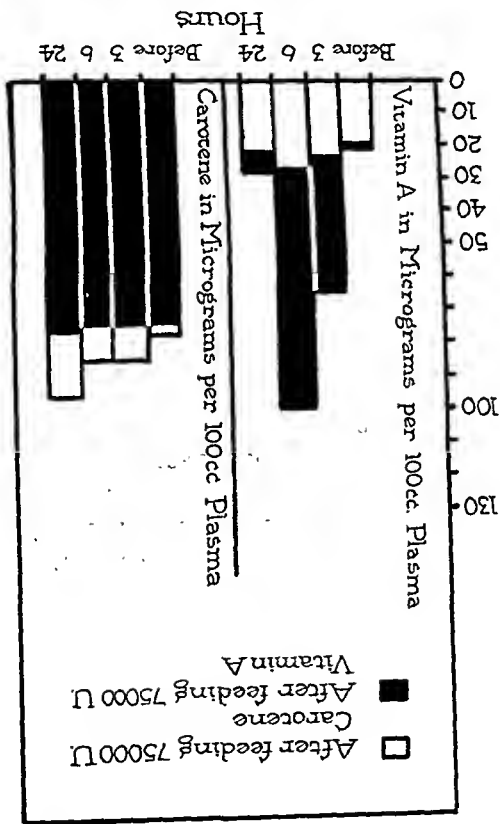


Fig. 4.—Averages of plasma vitamin A and carotene levels before, 3, 6 and 24 hours after ingestion of carotene equivalent to 75,000 I.U. vitamin A in 11 cases (white columns), compared to the averages of the tolerance curves following the ingestion of 75,000 I.U. vitamin A (white plus black columns).

**Discussion.** It might be anticipated that the response of the plasma vitamin A level to the intake of high doses of vitamin A should run parallel to the height of the initial plasma vitamin A level, since both plasma vitamin A level and tolerance curve evidently depend upon the intestinal absorption of vitamin A. The influence of the nutritional intake of vitamin A upon the plasma level is well established,<sup>21, 23, 28, 29, 33, 48</sup> and the relation between intestinal absorption and tolerance curve has recently been emphasized.<sup>5, 37</sup> Our findings, however, show that the plasma level and the maximal increase in the tolerance curve are parallel only when the former is high.

The usual lack of parallelism between the plasma vitamin A level and the maximal increase in the tolerance curves may be partly explained by the fact that the plasma vitamin A level depends not only upon the intestinal absorption but also on the ability of the liver to regulate the blood vitamin A level.<sup>1,35,38</sup> Thus, in healthy individuals the plasma vitamin A level may be maintained for some time even if no vitamin A is taken,<sup>7,35,45</sup> while in patients with acute liver damage the plasma vitamin A level drops quickly despite a sufficient intake of vitamin A.<sup>8,30,34,38</sup>

Another explanation for the lack of parallelism between the plasma vitamin A level and the rise of the tolerance curve are abrupt changes in intestinal absorption, *i. e.*, changes in absorption will influence the shape of the tolerance curve speedier than the level of the plasma vitamin A. Thus, in the initial stage of liver damage, the intestinal absorption may be almost completely inhibited while the blood level has not quite reached zero. On the other hand, in the recovery stage from liver disease, absorption may already be normal while the plasma vitamin A level is still low.

A third factor for this lack of parallelism is variations in the oxidative destruction of vitamin A in the lumen of the intestine. Various experiments have shown that following the administration of high doses of vitamin A, only a small percentage can be accounted for.<sup>4,27</sup> If we consider a maximal increase of 100  $\mu$ g. per 100 cc. as normal and assume the plasma volume as 3000 cc., then the accounted-for vitamin A in the blood is 3000  $\mu$ g. or 9840 I.U. if a conversion factor of 3.28 is used.<sup>19</sup> This would represent only about 13.3% of the ingested vitamin A. A certain amount is doubtlessly stored in the liver since the amount determined in the blood is only that found during the transport. However, a destruction of vitamin A by oxidative processes should also be considered. Recently, vitamin B was considered to be an important antioxidant agent.<sup>5,18</sup> However, our attempt to increase the height of the tolerance curve by administration of 50 mg. of alpha-tocopherol was unsuccessful. Further attempts with variations in the dose of tocopherol seem indicated.

In the search for causes responsible for variations in the tolerance curve, the total lipid concentration of the blood was discarded as a factor. Similarly the blood cholesterol level does not seem to have any relation to the height of the tolerance curve.

However, the form in which the vitamin A is administered is of importance. Thus, if it is given in the form of the provitamin beta-carotene, the plasma vitamin A and carotenoid level hardly change. The low response in the tolerance curve coincides<sup>9,12,35,46</sup> with some human<sup>4,8,12,27</sup> and animal<sup>10,41</sup> experiments. The question may arise whether this difference is due to destruction of carotene in the intestine, inability of the intestine to absorb carotene, or immediate liver storage of the carotene before the blood level rises. The fact that, of enormous doses of carotene a much smaller part is probably absorbed than of similar doses of vitamin A, does not necessarily imply that the same

difference prevails after intake of the much smaller doses of carotene contained in the food.

However, whether the vitamin A is taken as an alcohol or in the esterified form does not influence the shape of the tolerance curve, which observation agrees with experiments on rats.<sup>14,16</sup> This finding is significant, since vitamin A is absorbed as alcohol and esters are split during absorption.<sup>17</sup> Difficulties in the vitamin A esters hydrolysis are not an important factor in the shape of the tolerance curve.

The route by which vitamin A is administered plays a part in the shape of the tolerance curve.

The question of parenteral supply of vitamin A is important in view of the fact that many patients, especially in liver disease, are almost unable to absorb vitamin A administered orally.<sup>6,39,42</sup> However, following the intramuscular injection of 75,000 I.U. vitamin A, we did not observe a rise in the plasma vitamin A level. The fact that the slight rise in the plasma vitamin A level encountered in few cases usually disappeared within 24 hours, speaks against the possibility that delayed absorption accounts for this lack of response in the tolerance curve. Rourke and Stewart<sup>44</sup> gave intramuscular injections of 500,000 I.U. to patients with obstructive jaundice. They likewise failed to note a rise in the plasma vitamin A level, but in subsequent biopsies found relatively higher liver stores in these than in untreated cases. This would imply that, despite a lacking rise of the plasma vitamin A level, the liver stores of vitamin A are increased. However, in view of the impaired release of vitamin A from the liver in hepatic diseases<sup>34,40</sup> the intramuscular administration of vitamin A is of doubtful value since, apparently, only the vitamin A which circulates in the plasma is of functional significance. The relative ineffectiveness of the intramuscular injection of vitamin A in oil, as compared to the oral intake which was also found in various animal and human experiments,<sup>3,13,25,41,47</sup> may be due to the vehicle used. The injection of vitamin A to animals in aqueous colloidal preparations<sup>26</sup> or propylene glycol<sup>3</sup> results in improvement of vitamin A deficiency signs as well as in increased liver stores.

**Summary.** This study deals with several factors which may influence the response of the plasma vitamin A level to the ingestion of high doses of vitamin A (tolerance curve).

1. The shape of the tolerance curve is not necessarily related to the fasting plasma vitamin A level. This indicates that the plasma vitamin A level depends not solely upon current efficiency of the intestinal absorption for vitamin A.

2. The administration of the antioxidant vitamin E (in the given amount) did not influence the tolerance curve.

3. No apparent relation was found between the fat and cholesterol concentration in the blood and vitamin A tolerance curve.

4. Ingestion of vitamin A alcohol or vitamin A esters causes a similar rise of the plasma vitamin A level in the tolerance curve, whereas doses of carotene only have a minimal effect.

5. Intramuscularly administered vitamin A does not appreciably raise the plasma vitamin A level.

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## A METHOD FOR STANDARDIZING PENICILLIN

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The use of penicillin in the treatment of infections is accompanied by a number of technical problems not common to other therapeutic agents. One of the difficulties results from the tendency for the potency of penicillin to decrease under a variety of conditions. At the present stage of development of penicillin there is often a wide discrepancy between the estimated strength of a product at the time it is prepared and the actual strength at the time of use. This is not surprising in view of the great lability of penicillin. In some instances these differences appear to be due to the method of assay employed. Because of the scarcity of penicillin and the difficulties involved in its manufacture, it is important not to use more of it in the treatment of individual patients than is necessary; likewise it is most important to use enough. It is desirable, therefore, to be able to determine the strength of a preparation of penicillin at the time it is being administered.

One of the standard methods for assay of penicillin is that described by Abraham and co-workers.<sup>1</sup> It is known as the Oxford method and the Oxford unit is generally used to express the potency of a preparation even when it has been standardized by methods other than the Oxford method. Broth dilution methods employing serial dilutions of penicillin may be used for rough determinations of potency, but because the differences between concentrations are twofold, the error is necessarily great. In the turbidimetric method of Foster<sup>2</sup> the differences between concentrations of penicillin used are relatively small, and the accuracy of the method is said to be within  $\pm 10$  or 15%. Foster and Woodruff<sup>3</sup> have recently compared other methods of assay with the turbidimetric method.

Methods sufficiently sensitive for this purpose require the use of a preparation of penicillin of known potency as a standard in individual tests. One of the chief hazards in the assay of penicillin is the maintenance of a suitable standard. Under ordinary conditions the standard must be compared with other standards from time to time to be sure that it has not decreased in potency.

A method in use in this laboratory has been found reliable when compared with other methods; furthermore it does not require the use of a reference standard in individual tests. A determination is made of the amount of penicillin that will prevent the growth of a strain of *Diplococcus pneumoniae* in semisolid tissue culture medium. The method is an adaptation of one described by King, Henschel and Green in their studies on sulfonamide compounds.<sup>9,10</sup> It has been used in comparative studies on the bacteriostatic activity of antibacterial agents of microbial origin and germicides.<sup>4,6</sup> The pneumococcus was chosen as a test organism because it grows well in the

medium employed, it is sensitive to the action of penicillin and it does not liquefy the plasma clot, thus making possible an accurate colony count throughout the incubation period.

# Experimental.

The general laboratory procedures followed were similar to those described by King for tissue culture technique.<sup>7</sup>

## Tissue Culture Medium.

Healthy adult male rabbits were used exclusively as a source of blood for the preparation of medium. Rabbits were not fed during the 24 hour period before they were used. Blood was obtained under sterile conditions by cardiac puncture or by carotid cannulation with the animal under light ether anesthesia. Serum was prepared and heparin was added to a portion of the blood for the preparation of plasma. The solution of heparin used contained 0.04% of purified heparin (Connaught Laboratories) and 0.8% of sodium chloride. This solution was sterilized by Berkeley filtration and 0.25 cc. was used for 15 cc. of blood. Tubes containing blood to which heparin had been added were thoroughly chilled before being placed in the centrifuge. After the blood had been centrifuged the plasma was transferred to a sterile tube and packed in ice. Serum and plasma showing abnormal cloudiness or opalescence, or a considerable degree of hemolysis, were discarded. A slight degree of hemolysis did not appear to affect the results. A serum-chick embryo extract was prepared by extracting chick embryos of 8 days of incubation with rabbit's serum in the proportion of 1 embryo to 5 cc. of serum.

## Bacterial Cultures.

A strain of *Diplococcus pneumoniae* Type 7 originally isolated from exudate from an empyema cavity was used as the test organism. Cultures were grown in Hartley broth containing approximately 10% of horse serum. When a culture had attained maximal growth, suitable dilutions were made in Hartley broth and added to the serum-chick embryo extract in the proportion of 1 part of the bacterial suspension to 40 parts of serum extract. The final concentration of the original bacterial culture in the tissue culture clot was approximately 1:10,000,000. This inoculum usually resulted in the appearance of 20 or more bacterial colonies in each preparation. It was found that a more uniform bacterial count could be obtained on different days by using a suspension of pneumococci stored in divided amounts in solid carbon dioxide than when freshly grown cultures were used. Tubes containing tissue extract to which pneumococci had been added, as well as tubes containing plasma, were kept chilled throughout the experiment. It was found to be important to use freshly prepared extract for each day's determinations. Some variation resulted when plasma and extract that had been prepared the day before were used.

## Penicillin.

The penicillin used to standardize the method was a preparation of the sodium salt of penicillin obtained from the Northern Regional Research Laboratories of the United States Department of Agriculture through the courtesy of Dr. Robert Coghill. By recent comparison with other standards by means of the Oxford method its potency had been determined as 28 Oxford units per mg. Additional tests were carried out with a standardized preparation of the calcium salt of penicillin obtained through the courtesy of Dr. Coghill. The potency of this preparation was 135 Oxford units per mg. Twelve other preparations from 5 different sources were tested (Tables 1 and 2). As soon as they were received, individual samples were weighed under sterile conditions and added to 0.8% solution of sodium chloride to make a concentration of 1:500. These stock solutions were stored in divided amounts in solid carbon dioxide until they were needed for the preparation of suitable dilutions in 0.8% solution of sodium chloride at the time the tests were made. Experiments with 1 sample of penicillin were usually carried out within a period of a few weeks, as prolonged storage of the stock solutions in solid carbon dioxide sometimes resulted in a loss of potency.

In instances in which the potency of penicillin received was expressed as the number of Oxford units per ampule and the total weight of the sample was not

TABLE 1.—ASSAY OF PENICILLIN

Source of penicillin	Sample No.	Test No.	Colonies per culture (controls)	Concentration of penicillin		Assay at source (Oxford units/mg.)	Assay, present method (Oxford units/mg.)	Reassay at source (Oxford units/mg.)
				Causing total inhibition (µg./cc.)	Not causing total inhibition (µg./cc.)			
A	1	1	60	1.00	0.50	98	84	125
		2	100	1.00	0.90			
		1	20	0.50	0.25			
		2	36	0.50	0.40			
		3	15	0.50	0.40			
B	2	1	20	0.50	0.25	138	210	
		2	12	0.40	0.30			
		3	36	0.40	0.30			
		4	22	0.40	0.30			
		5	21	1.00	0.50			
	3	1	24	1.00	0.50	200	262	
		2	15	0.60	0.30			
		3	60	0.32	0.16			
		4	16	0.32	0.16			
		5	26	0.41	0.40			
C	1	1	25	0.41	0.41	215	205	
		2	32	0.40	0.40			
		3	20	0.40	0.36			
		4	24	0.46	0.50			
		5	28	0.62	0.59*			
	2	1	26	0.59	0.56	250	142	146
		2	24	0.59	0.56			
		3	26	0.59	0.56			
		4	32	0.59	0.56			
		5	150	0.59	0.56			

\* One colony only in all 4 cultures at this dilution.

TABLE 2.—ASSAY OF PENICILLIN IN TERMS OF OXFORD UNITS PER AMPULE

Source of penicillin	Sample No.	Test No.	Colonies per culture (controls)	Concentration of penicillin		Assay at source (Oxford units per ampule)	Assay, present method (Oxford units per ampule)	Reassay at source (Oxford units per ampule)
				Causing total inhibition (Oxford units/cc. *)	Not causing total inhibition (Oxford units/cc.)			
B	6	1	42	0.080	0.060	10,000	10,500	21,000
		2	150	0.080	0.060			
		3	42	0.080	0.060			
		4	22	0.107	0.094			
		5	24	0.107	0.094			
C	2	1	28	0.107	0.094	30,000	23,550	
		2	28	0.107	0.094			
		3	28	0.107	0.094			
		4	25	0.100	0.084			
		5	32	0.100	0.084			
D	1	1	32	0.250	0.166	10,000	8,400	
		2	32	0.250	0.166			
		3	32	0.250	0.166			
		4	20	0.250	0.166			
		5	20	0.250	0.166			
E	1	1	2	0.250	0.166	25,000	8,400	
		2	2	0.250	0.166			
		3	2	0.250	0.166			
		4	2	0.250	0.166			
		5	2	0.250	0.166			

\* Values are expressed in terms of original assay at the source. The end-point in this method is 0.084 Oxford units per cc.

known, the results of tests were expressed in terms of Oxford units per ampule instead of Oxford units per mg. (Table 2). The results of the individual tests were expressed in terms of the potency as determined at the source, and the final estimation of the value of the product was arrived at by multiplying the number of Oxford units per ampule as determined at the source by the fraction  $0.084$  Oxford units necessary to inhibit *Plasma Clot Cultures*. Portions of each dilution of penicillin to be tested were added to both plasma and serum-chick embryo extract at the time the

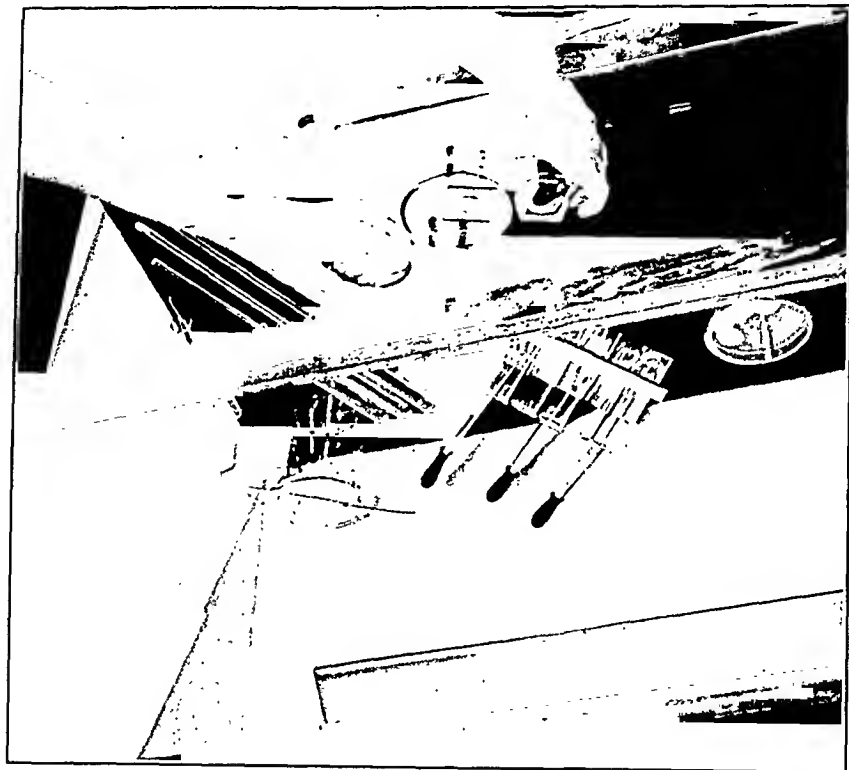


Fig. 1.—Preparation of cultures for the assay of penicillin. The photograph shows the arrangement of equipment in the culture hood. This view shows a plasma clot culture in a Petri dish just before it is to be sealed with a hollow ground slide. The other Petri dish contains sterile 22 mm. round coverslips. Tubes held at an angle of 45° in the rack are used for the following purposes: the first tube, from left to right, contains saline solution used to attach 22 mm. round coverslips to heavier square glass slides; in the second tube is used to receive discarded mixtures of plasma and penicillin prepared in the third tube; the fourth tube is used to receive discarded mixtures of serum extract containing pneumococci and penicillin which had been mixed in the fifth tube for the preparation of cultures. Two pipets of 1 cc. capacity and one pipet of 0.2 cc. capacity are used for measuring plasma, serum extract and solutions of penicillin respectively are shown with tips resting in sterile tubes.

cultures were made in order to insure a uniform concentration of penicillin throughout each culture. Since the amount of medium necessary for each group of cultures was small, 0.1 cc. portions of solution of penicillin were added to 0.9 cc. of plasma and serum extract respectively. Control cultures received a tenth by volume of 0.8% solution of sodium chloride instead of penicillin. The same mixing tubes and standardized pipets could be used for testing various concentrations of the same sample of penicillin by preparing cultures containing the least amount of penicillin first. Serum and plasma were



removed carefully from the mixing tubes after one group of cultures had been made and before medium was prepared containing a greater amount of penicillin. The preparation used in making the culture was a modified Maxmow type of preparation in which the culture was planted on a 22 mm. round cover slip attached by a small drop of saline solution to a heavier glass slide. With a standardized pipet 1 drop (approximately 0.05 cc.) of plasma containing penicillin was placed on the cover slip and evenly distributed by means of a sterile wire. Three drops of serum extract containing the test organism and penicillin were added to the drop of plasma on the cover slip and well mixed. Four cultures were prepared for each experimental condition. Each culture was covered with a sterile, hollow ground slide rimmed with petrolatum (Fig. 1). At the conclusion of the experiment cultures were sealed with a mixture of paraffin and petrolatum and were incubated at 37° C. in a specially constructed down-draft incubator. Final observations were made after approximately 36 hours of incubation. At that time the number of bacterial colonies in each culture was determined with the aid of a dissection microscope at a magnification of 7X. An estimate was made of the average number of colonies present in control cultures not containing penicillin. An end-point was chosen arbitrarily as the lowest concentration of penicillin that would completely prevent the appearance of bacterial colonies in all 4 cultures. This end-point was determined several times for each preparation if the size of the sample would permit. The initial test in each instance was usually done with concentrations of penicillin spaced more widely apart than in subsequent tests in order to determine the range of activity of the sample. Several different preparations were tested on the same day.

**Results.** It was found that the growth of the test organism was prevented by 3 µg. per cc. of the standard sodium salt of penicillin but not by 2.86 µg. per cc. Since the potency of this standard was 28 Oxford units per mg. the amount of penicillin necessary to prevent bacterial growth was presumably 0.084 Oxford units per cc. in the final tissue culture clot. Using this value the potency of each of the other preparations was determined by dividing 84 by the number of micrograms of each sample necessary to cause total inhibition. In tests done with a second standard, a calcium salt of penicillin, 0.59 µg. per cc. caused total inhibition of bacterial growth; thus the potency as determined by this method was 142 Oxford units per mg. This result showed an agreement of within 5% with the original assay of potency of 135 Oxford units per mg. The results of individual tests with preparations of penicillin from different sources appear in Tables 1 and 2. Similar results were usually obtained on different days with the same sample of penicillin, particularly when widely spaced concentrations of the drug were used as in earlier tests. When there were smaller differences between concentrations used, as in the case of sample B5, varied results were obtained; however, the variation in this instance was within a range of 10%.

The difference between the results of assay of a product at the source and the value determined by this method was sometimes very great. All samples showing a greater potency by the present method than that claimed by the manufacturer were obtained from one source. In the case of 2 samples from source C the material was tested again at the source by the Oxford method and the results showed good agreement with the results obtained in this laboratory. Results of tests on

preparations A and D did not differ more than 16% from the results obtained at the source. Sample B was apparently less than half as potent as the estimate at the source would indicate.

**Comment.** The disadvantages of the method described here are those common to tissue culture technique in general, and no attempt will be made to minimize the difficulties to be overcome where tissue culture facilities are not already available. The method does offer certain unique advantages not only for the assay of penicillin but also for quantitative studies on other antibacterial agents. Experience with the use of the tissue culture preparation in bactericidal tests over a period has resulted in the conclusion that certain Gram-positive cocci grow with remarkable regularity when cultivated under these conditions. Tissue culture mediums prepared in a similar fashion appear to be more uniform than the usual bacteriologic mediums. The same concentration of penicillin tested at different times regularly caused the same degree of inhibition of bacterial growth. Relatively small amounts of penicillin are sufficient for the performance of a number of tests. Antibacterial agents less diffusible than penicillin may also be studied and compared by this technique.

This method is not recommended for determining the amount of penicillin in a patient's blood during treatment, as the presence of human serum in cultures decreases the accuracy of the results. Fortunately it is not necessary to know the concentration of penicillin in the blood in order to obtain good therapeutic results. It has been found that when adequate amounts of penicillin are given by almost continuous intravenous administration good results may be obtained in spite of the fact that penicillin cannot be detected in the blood by broth dilution methods.<sup>5</sup> It is much more important to know how much penicillin the person under treatment is receiving than to be able to detect the presence of penicillin in the blood by methods available at the present time.

**Summary.** A method has been described for the standardization of penicillin in which a determination is made of the amount of penicillin necessary to prevent the growth of pneumococci in semisolid tissue culture medium.

The principal advantage of this method is that it is not necessary to use a standard preparation of penicillin in the performance of the test. The amount of penicillin needed for a determination of potency is small. The accuracy of the method appears to compare favorably with that of the Oxford method in a limited number of tests. Repeated tests with the same sample of penicillin indicate that the variation of the results obtained is fairly small.

Preparations of penicillin from different sources have been standardized by this method. Results obtained as well as tests done elsewhere indicate that the actual potency of a product may be significantly different from the estimated potency at the time of preparation.

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## SULFANILAMIDE AS A PROPHYLACTIC MEASURE IN RECURRENT RHEUMATIC INFECTION

### A CONTROLLED STUDY INVOLVING 131 "PATIENT-SEASONS"

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PREVIOUS reports suggest that sulfanilamide is of value as a prophylactic agent against recurrences of rheumatic fever. In August, 1942, Thomas<sup>1</sup> summarized the work of a number of clinics in the use of small, daily doses of sulfanilamide. In that series, the recurrence rate was less than 1% among several hundred treated patients. Since then, Hansen, Platon and Dwan<sup>2</sup> have reported similarly favorable results. Because of the vagaries of rheumatic fever, the experience of any one clinic may be distorted. Geographic differences must be evaluated, and it is important, therefore, to consider results from many different regions. The present report, which confirms the observations of other investigators, should be appraised, not alone, but as an addition to the accumulated data. Toxic manifestations were not severe, and they will be discussed in detail.

**Method.** A program of prophylaxis was undertaken among out-patients of the Cardiac Clinic of Milwaukee Children's Hospital during two seasons (1941-42 and 1942-43). The method of control was designed to insure random selection as far as possible. Patients had previously been assigned in rotation to a clinic held on Monday, Wednesday or Saturday, and in most instances they had visited the same clinic for months or years. Children with a history of definite rheumatic fever or chorea and those with undoubted rheumatic heart disease, even though there was no positive history, were included in the study. All such patients in the Monday or Saturday clinics received sulfanilamide, and those in the Wednesday clinic acted as controls. In every instance, the rheumatic process was regarded as quiescent when the study began. The season extended from October or November to May or June. The period of observation averaged 5.5 months each season for patients in the control group and 6.3 months for those who received sulfanilamide. Some

children dropped out of both groups before they had been in the study as long as 4 months, and they have not been counted in this report. None of those who discontinued the study developed recurrences while under observation. Patients receiving sulfanilamide came to the clinic every 3 or 4 weeks, and those in the control group appeared at slightly longer intervals. In addition to history and physical examination, the erythrocyte sedimentation rate, leukocyte count, hemoglobin and throat culture were obtained at each visit. The blood sulfanilamide level was determined in those who were receiving the drug. Electrocardiograms, Roentgen rays, and other laboratory work were requested when indicated.

There were 32 children in the control series, and some were in this group both years, so that, altogether, 42 "patient-seasons" are represented. The treated group consisted of 69 patients comprising a total of 89 patient-seasons during the 2 years. Among those who received no medication, there were 15 boys and 17 girls; the range of age was 4 to 14 (average, 8.6 years). There were 39 boys and 30 girls in the treated group; the ages ranged from 4 to 15 (average, 9.5 years).

Most patients were given 15 gr. (1 gm.) of sulfanilamide daily in three divided doses. At times, as much as 30 gr. (2 gm.) or as little as 5 gr. (0.3 gm.) daily were used, depending on the blood sulfanilamide level and the presence of toxic symptoms. An attempt was made to maintain the level between 1 and 3 mg. per 100 cc, but it ranged from 0 to 6.6 mg.

**Results. Rheumatic Recurrences.** During the two seasons, none of the treated children developed a recurrence of rheumatic fever. Thomas *et al.*<sup>5</sup> have appropriately defined a major rheumatic episode as an illness lasting at least a week, in which fever, polyarthritis, active carditis, chorea, or other signs are unequivocal. A minor rheumatic episode is characterized by transiently painful, swollen joints, or mild choreiform movements. There were 3 (7.2%) major and minor rheumatic episodes among the 42 control patient-seasons (Table 1).

The one major recurrence affected a girl of 14, who had suffered two previous recurrences following her first attack of rheumatic fever in 1935. She had well-established mitral insufficiency. She was discharged from the Convalescent Home in June, 1941 and entered the control group in October, 1941. The first season she enjoyed unusually good health, and she was well at the start of the second season. A sore throat late in December, 1942 was accompanied by fever and pain in both knees for several days. Although she was not seen until 2 weeks after this episode, it was regarded as recurrent rheumatic fever. She was kept in bed from January to April, 1943, and during this time tachycardia, anemia, failure to gain weight, and persistent elevation of the sedimentation rate were observed.

Both minor recurrences developed during the first season. One affected a girl of 6, who had mitral insufficiency but no definite rheumatic history. In December, 1941, and January, 1942, she showed choreiform movements and an elevation of the sedimentation rate. These signs were absent the remainder of the year and the following season.

The other patient was a boy of 8, who had mitral insufficiency without a definite history. In January, April and May of 1942, he complained of nervousness and was observed to manifest definite choreiform movements.

Thomas *et al.*<sup>5</sup> have classified a questionable rheumatic episode as any illness in which the diagnosis of rheumatic fever is in doubt as well as such symptoms as epistaxis, sore throat, arthralgia, or pre-cordial pain. Among control patient-seasons, 13 (31%) experienced 23 questionable episodes. Of the children treated, 19 (21.4%) presented 23 such incidents (Table 1).

TABLE 1.—RESULTS OF SULFANILAMIDE PROPHYLAXIS

42 control patient-seasons		89 treated patient-seasons	
No.	%	No.	%
1	2.4	0	0
2	4.8	0	0
13	31.0	19	21.4
Total		16	19
Initially positive beta hemolytic streptococcus throat cultures		17	41
Subsequent throat cultures		157	546
Subsequent positive throat cultures		76	223
Total		16	40.8

*Throat Cultures.* Comparative results are shown in Table 1. At the start of each season, 17 (40.4%) of the control patients and 41 (46.1%) of the children who were to receive sulfanilamide showed positive throat cultures for the beta hemolytic streptococcus. Among the untreated subjects, 157 subsequent cultures were made and, of these, 76 (48.4%) were positive. In the other group, 546 cultures were obtained after treatment had begun, and 223 (40.8%) were positive. In order to determine the effect of the blood sulfanilamide level, a separate analysis was made of the children who consistently had levels of 1 mg. or more. Thirty-one patient-seasons were represented in this subgroup, and 87 (43.5%) of 200 cultures were positive. None of the differences is statistically significant.

This experience is greatly at variance with the observations of Thomas, France and Reichsmann,<sup>5</sup> who found that giving sulfanilamide practically eliminated positive beta hemolytic streptococcus throat cultures. Chandler and Tauszig<sup>6</sup> found no major difference in the incidence of positive throat cultures between patients in the treated group and those who acted as controls.

*Evidence of Toxicity.* Toxic symptoms were infrequent and unimportant, as shown in Table 2. Every symptom which could possibly be regarded as evidence of sulfanilamide intoxication has been included. Only 12 (11.2%) of the 107\* children who started to take sulfanilamide showed symptoms, and in none was a leukopenia associated. In 2 instances, the drug was permanently discontinued (B.K. and P.D.). In 3 (M.M., C.O., and C.R.) it was temporarily stopped, and in the remainder it was continued at the same or reduced dosage.

A fall in the white blood cell count below 4500 per c.mm. was observed in 10 (9.2%) patient-seasons. None of these children had subjective complaints suggestive of toxicity. No severe degrees of leukopenia were observed. Details are shown in Table 3. Leukopenia

\* This figure includes 18 patient-seasons in which sulfanilamide was used for less than 4 months.

was not observed prior to the second visit, *i. e.*, on the 42d day of treatment. In some cases, it did not appear until as late as the 75th or 101st day of treatment. In 2 patients (R.Z. and J.K.) sulfanilamide was permanently discontinued. In 4 (P.V., N.M., T.B., and D.H.2) it was temporarily stopped, and in the others it was continued at the same or a reduced dose. It is interesting that 2 patients (D.H. and F.M.) showed leukopenia both seasons.

TABLE 2.—SYMPTOMS SUGGESTIVE OF SULFANILAMIDE TOXICITY

Patient	Sex	Age (yr.)	Daily dose (gr.)	Symptoms	Day level of (mg./100 cc.)	Blood level	Subsequent course	Experience other year
B. K.	F	3	15	Transient itching rash of arms; patient's mother discontinued sulfanilamide; rash not observed in clinic	7	..	This was early in our experience and 15 gr. of sulfathiazole daily substituted for balance of year	0
P. Do.	M	11	15	Patient uncooperative; nausea and vertigo prompted parent to discontinue drug and withdraw child from study	60	0.2	Previous blood levels 0 and 0; patient not regular in taking drug	Uncooperative
M. M.	F	9	15	Diarrhea and vertigo for 3 days; patient's mother discontinued drug	120	0.9	Drug resumed with no ill-effects	No difficulty
C. O.	M	8	10	Skin dry and scaly; hemoglobin 10.5 gm.	133	2.7	Drug discontinued for 2 wks.; then resumed with no further difficulty	1 yr. 15 gr.
C. F.	M	10	15	Somnolence; this continued even when dose was reduced to 10 gr.	60	2.9	Symptoms then entirely relieved by 3 wks. discontinuance of the drug; minor degrees of drowsiness balance of year on doses of 10 or 15 gr.	1 yr.
G. C.	M	12	15	Nausea; patient's mother reduced dose to 10 gr.	30	1.7	Dose again raised to 15 gr., abdominal discomfort resulted; dose reduced to 10 gr., where it remained	No difficulty on dose of 15 to 30 gr.
D. C.	M	6	20	Moderate nausea and vomiting	112	5.9	Dose reduced to 10 gr. with no return of symptoms	1 yr.
P. M.	F	7	15	Slight nausea and vertigo	28	0.5	Dose reduced to 10 gr.; 4 wks. later again complained of nausea; same dose (10 gr.) continued without further difficulty	1 yr.
R. B.	M	4	10	Nausea, vertigo, somnolence	140	3.4	Previous sulfanilamide levels of this height had not been associated with symptoms; dose reduced in 5 gr. with no further ill-effects	1 yr.
P. D.	F	6	15	Nausea for first 2 or 3 days	1	2.7	Dose kept at same level and no further complaint	1 yr.
M. S.	F	13	20	Slight nausea for 1 day	40	0.4	Same dose continued; again slight nausea on 153d day when sulfanilamide level was 0.3; no subsequent difficulty with blood level	No difficulty on dose of 15 gr.
D. V.	F	10	15	Nausea for 3 days; faint macular rash with axillae brownish cast of both	21	0.5	Same dose continued without further trouble	1 yr.

TABLE 3.—LEUKOPENIA

Patient	Sex	Age	Daily dose (gr.)	Blood picture	Day level of (mg./100 cc.)	Subsequent course	Experience other year	
H. Z.	M	8	15	WBC 4400 Granulo- cytes 56%	42	1.6	Same dose continued; increased to 20 gr. 4 wks. later when WBC 5050 and blood level 0.5 mg.; 6 wks. later WBC 3250, granulocytes 36%; blood level 2.8 mg.; dose of 20 gr. continued; 4 wks. later WBC 3450, granulocytes 56%; sulfathiazole in daily doses of 15 gr. substituted for balance of season; no further leukopenia	1 yr.
J. K.	M	12	15	WBC 3900 Granulo- cytes 49%	49	1.7	Continued at same dose; no leuko- penia until 3½ mos. later when WBC 3900, granulocytes 28%; drug discontinued and WBC rose to 4800 with 43% granulocytes 2½ mos. after drug withdrawn	1 yr.
P. W.	M	4	15	WBC 3950 Granulo- cytes 21%	42	2.7	Drug discontinued 1 wk.; resumed at dose of 15 gr.; later varied from 5 to 10 gr., depending on blood sul- fanilamide level; no return of leu- kopenia	1 yr.
N. M.	M	12	20	WBC 4300 Granulo- cytes 47%	56	1.7	Drug discontinued for 1 wk. after kopenia	1 yr.
T. B.	M	5	10	WBC 3750 Granulo- cytes 39%	56	0.1	Drug discontinued 3 wks. after leukopenia	Dose 10 gr.; no leukopenia; av. blood sul- fanilamide level 2.7 mg.
D. H. 1	M	7	10	WBC 3500 Granulo- cytes 51%	42	1.8	Dose of 10 gr. continued; 4 wks. later with no leukopenia	Leukopenia fol- lowing year;
D. H. 2	M	8	10	WBC 3900 Granulo- cytes 27%	56	2.9	Drug discontinued; resumed 4 days later; 5 wks. later WBC 7900; 4 wks. later, dose increased to 15 gr.; no leukopenia	Leukopenia; see below
F. M. 1	M	5	15	WBC 4400 Granulo- cytes 23%	75	0.2	Dose of 15 gr. continued; 4 wks. later sulfanilamide level 0.2 mg.	Leukopenia fol- lowing year;
F. M. 2	M	6	15	WBC 4400 Granulo- cytes 55%	63	0	Dose reduced to 10 gr. thereafter; this episode 1.1 to 3.3 mg. blood level prior and subsequent to used after with no leukopenia;	Leukopenia; see above
C. G.	M	10	20	WBC 4100 Granulo- cytes 39%	101	2.1	Lost contact with this patient	No leukopenia

Some degrees of anemia were observed in both groups with the hemo-  
globin occasionally falling as low as 10.5 gm. Anemia was no more  
frequent among treated than control patients. The administration of  
ferrous sulphate satisfactorily controlled the anemia in every case.

Discussion. This series of 89 sulfanilamide treated patient-seasons  
without a recurrence takes on greater significance when it is added to  
the other favorable reports in the growing literature. The relatively  
low incidence (7.2%) of recurrences among control patients is difficult  
to explain. In the light of previous experience with rheumatic fever in  
Milwaukee, this favorable relapse rate was unexpected. Major, minor  
and questionable rheumatic episodes combined occurred in 13 (38.2%)  
of the control patients as compared with 19 (21.4%) of the treated  
children. The difference is statistically significant and justifies the

conclusion that the drug was beneficial. Sulfanilamide did not affect the incidence of throat cultures positive for the beta hemolytic streptococcus. The reason for this variation from the findings of Thomas, France and Reichsmann<sup>5</sup> is not evident. Further study is indicated. It now seems clear that sulfanilamide can be given in small, daily doses over long periods without serious ill effects. Suggestively toxic symptoms or leukopenia made it necessary to discontinue sulfanilamide permanently in only 4 (3.7%) of 107 patients who started taking the medication. This confirms the results of all other workers except Stowell and Button.<sup>3</sup> Using somewhat larger doses, they observed one death due to agranulocytosis and a number of less severe toxic reactions. Summary. Sulfanilamide in small, daily doses was given to rheumatic children in the Cardiac Clinic of the Milwaukee Children's Hospital during the autumn, winter and spring of 1941-42 and 1942-43. Altogether, 89 patient-seasons are represented in the treated group and 42 patient-seasons in the control series. No rheumatic recurrences appeared among the children who took sulfanilamide. There were 3 (7.2%) major and minor recurrences among control patients. Questionable rheumatic episodes were observed with greater frequency in the control group. The incidence of positive beta hemolytic streptococcus throat cultures was approximately the same in the two groups. Manifestations of sulfanilamide toxicity were neither frequent nor severe. Sulfanilamide is recommended as a relatively safe and effective prophylactic measure against recurrent rheumatic infection.

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## A STUDY OF SULFONAMIDE AEROSOL INHALATION

### A SUPPLEMENTAL NOTE

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The utilization of the respiratory tract as a route of administration of drugs, notably the sulfonamides, and the effects produced by inhalation of these drugs in the therapy of upper respiratory tract infections,



are the subjects of the present report. An earlier paper<sup>3</sup> dealt with sulfonamide concentrations in the blood of mice which had inhaled suspensions of the drugs. Technical difficulties in achieving uniform blood levels by this method led to experimentation with smokes composed of crystals.

The purpose of this study was to determine whether or not: (1) inhalations of the drug would be the equivalent of topical application in the respiratory tract, supplying a high concentration of the drug to the infected site; (2) this might prove so efficient that a high level of the drug throughout the body would not be necessary; and (3) absorption of the drug would occur. These 3 questions appear to have been answered in the affirmative.

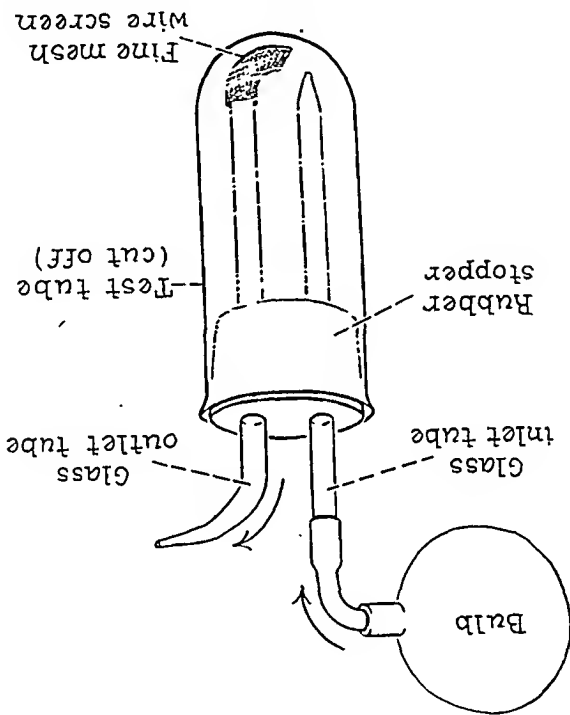


Fig. 1.—Simple type of powder blower for microcrystalline sulfadiazine.

**Method.** To produce the smoke efficiently from such cohesive powders as the microcrystalline sulfadiazine, it was necessary to make special powder blowers. The simplest of the many types used is shown in Figure 1. Essentially, it is a small bottle with an inlet arranged to blow across the mouth of an outlet tube, which latter is covered by fine-mesh wire screen. An atomizer bulb supplies a sufficient airstream. The blower contains the sulfonamide powder diluted with sea sand. The drug adheres to the sand and on agitation by the airstream is freed gradually from it as the sand is dashed about. This results in a fine smoke from which the sand is filtered by the screen on the outlet tube. The sand's activity and the direction of the inlet airstream keep the screen clear.

This device could not be employed in the experimental work on animals, since its concentration of smoke fell off logarithmically with the quantity of the drug remaining in the blower. For this reason a blower with a constant

feeding device was employed. However, for clinical purposes, the simple blower was suitable and gave satisfactory uniformity of blood levels.

The crystals employed in this investigation included the microcrystals of sulfadiazine and sulfathiazole and the macrocrystals of these sulfonamides and of sulfapyrazine. Their sizes were not constant, but the average diameter of the microcrystals of sulfadiazine used was approximately 2 micra, of the microcrystals of sulfathiazole, approximately 4 micra, of the microcrystals of sulfapyrazine, approximately 3 micra, of the macrocrystals of sulfathiazole, approximately 50 micra.

Attempts were made to determine the depth of penetration of the crystals into the lung in order to determine the point of their absorption. Lung preparations dried by means of blowing an airstream into the trachea of the excised lung showed high drug concentrations in the lung periphery but failed to define their source, *i. e.*, whether in air, lymph, or blood channels. After substituting various materials, including dyes, for the crystals in the inhaled air, it was found that carbon particles of comparable size were visible on sectioning the lung. Those smaller than 5 micra in diameter were seen in large numbers in the bronchioles and in the alveoli, as would be expected from the work of Bloomfield.<sup>1</sup> The particles were detected in the air spaces only. Sulfonamide smoke was administered by passing it through a dynamic chamber containing the animals to be treated. For a single mouse a small bottle was utilized as a chamber, and for large numbers, a rubber-gasketed box was used. Atmospheric pressure surrounding the animal was never above normal. The problem of absorption through the gastro-intestinal route was discussed previously, but it might be mentioned here that the mice had little tendency to lick themselves, probably because so few crystals were deposited on them. The sulfonamide levels were determined according to the microchemical method of Bratton and Marshall<sup>2</sup> on blood taken from the tail after careful cleansing with water and alcohol separately. The duration of the exposures to the smokes was at first several hours. This time was decreased to 10 minutes after the early experiments and later reduced to 5 minutes, which exposure was adopted as routine. Concentrations of the smoke were varied by employing quantities of 100 to 300 mg. of the drug to 5 gm. of the sand. Blood was taken immediately after the exposure (which time was never greater than 15 minutes after the animal left the chamber) and at stated intervals thereafter.

To determine the possible local changes resulting from inhalation of the sulfonamide crystals, rats, guinea pigs, and mice were used. They were subjected to various concentrations of smoke for periods varying from 5 minutes to 9 hours. The histologic picture even after the longest of these exposures showed nothing more than a slight hyperemia, which was no more than might have been produced by sacrifice. The mortality rate of those exposed animals which were otherwise well, was not increased over a period of 2 months. Drug concentrations in the blood were reached with approximately the same rapidity in all species used. Blood levels were found to be comparable in normal rats and in others whose noses were closed by sutures. A similar study on mice gave similar results.

Attempts to produce sensitization by inhalation of the crystals were made unsuccessfully in guinea pigs, of which 6 were subjected to the sulfonamide because the type of sensitization that occurs in man has never been reproduced in animals. One hundred and seventy-three mice were divided into groups of 1 to 20 in number and exposed for 5 minute periods every 3rd day for 2 to 4 weeks. Blood levels were determined on 2 or more of each group at the later described intervals.

Significant differences between the blood concentrations established by the various sulfonamides were not detected; usually the smaller crystals gave higher immediate blood levels.

The bloods taken within 15 minutes of a 5 minute exposure showed 3.4 to 7 mg. of the drug per 100 cc. of blood. Ten minute exposures produced concentrations of the same order of magnitude; 30 minute exposures produced 12 to 18 mg. per 100 cc.; 1 hour exposures, 18 to 22 mg. per 100 cc.; and 2 hour exposures 50 mg. per 100 cc. The mice having a longer exposure time, obviously had a longer absorptive time. In those mice subjected to an exposure of 10 minutes or less, the concentration in the blood climbed for approximately 6 hours to a point nearly double that of the immediate value. In 24 hours, the level fell to 75% of the immediate value and in 48 hours, it was one-half of the immediate value. The concentration of the smoke, hence the blood levels, varied with the amount of drug employed in the blower. For example, a mouse exposed for 5 minutes to the smoke from a blower which contained 100 mg. of the microcrystalline sulfadiazine had a blood level of 14 mg. per 100 cc. in 1½ hours; another exposed similarly but with 200 mg. in the blower, had a blood level at the same time of 28 mg. per 100 cc., while a third with 300 mg. revealed a blood level of 39 mg. per 100 cc.

The sulfonamides have been administered by this route to approximately 100 persons with various bacterial infections of the respiratory tract. Accurate evaluation of this therapy is not possible at this time. However, several points of interest have presented themselves. First, there has been no evidence of drug reaction although asthmatics were included in the series. Inhalation of the large crystals produced a severe cough not unlike that seen with a foreign body in the lung. The cough lasted approximately 2 hours in the few cases in which large crystals were used. This cough, which was the only unfavorable symptom observed, was never noticed when the small crystals were employed. Second, an analgesic effect previously commented upon in experimental work with burns, was noted by many of the patients, particularly those with pharyngitis or laryngitis. This effect usually lasted 2 or 3 hours. Lastly, purulent nasal discharges frequently cleared in a few hours temporarily, and the duration of all symptoms appeared to be shortened. Blood samples from 5 volunteers were obtained from vein and finger-tip after warming the hand in water (Table I). The first determination was made within 30 minutes after the subject had inhaled deeply 3 breaths of sulfapyrazine from a blower. The values on any subject at a given time were within 1 mg. per 100 cc. of the others obtained at the same time.

TABLE 1.—SULFAPYRAZINE BLOOD LEVELS IN 5 VOLUNTEERS AFTER INHALATION

	½ hr.	5 hrs.	12 hrs.	18 hrs.	24 hrs.	48 hrs.
Venous blood	2.0	5.5	3.6	1.6	1.4	1.4
Finger-tip blood	3.5	..	..	2.5	2.6	1.5

Drug concentrations in the blood of one of us were determined on 8 occasions over a period of more than 6 months while these experiments were in progress, and the exposure to the smoke, while unintentional, was frequent. Determinations usually were made on Monday mornings after a day

away from the laboratory because sulfonamide-free blood was desired for other purposes. The drug levels, however, varied between 2 and 7 mg. per 100 cc. on every occasion.

**Discussion.** The sulfonamides are absorbed rapidly into the blood with little or no irritation visible on histologic sections of the lung of small animals. Whether the drug is absorbed directly by the capillaries or secondarily to them from the lymphatics is not clear. The second possibility is probably the more important one, as a reservoir seems to be maintained somewhere in the respiratory tract for a period of at least 6 hours.

Although in this series there is no evidence of sensitivity to the drug or other serious reaction, it is not suggested that this route has a detoxifying effect or that it is safer to administer sulfonamides by inhalation than by other routes. Instead, because of the rapidity with which high blood levels are attained, extra care should be used in the selection of patients in order to avoid any chance of drug reactions. **Summary and Conclusions.** The blood concentrations of the sulfonamides when administered as aerosols by inhalation and the treatment of upper respiratory tract infections by this method, have been presented. Crystalline sulfonamides, blown from a simple blower, were inhaled and their rapid appearance in the peripheral blood has been shown.

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## SULFADIAZINE AND ITS SODIUM COMPOUND IN TREATMENT OF MENINGOCOCCIC MENINGITIS AND MENINGOCOCCEMIA

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The object of this paper is to record the experience of the Service for Acute Infections of the Central Nervous System of the New York City Health Department with sulfadiazine and its sodium compound in the treatment of 141 cases of meningococcal meningitis. Of these, 139 recovered and only 2 died, a mortality of 1.4%. There were in addition 8 cases of meningococemia without evidence of meningococcal involvement, all of whom recovered. These patients were seen between Jan. 12, 1942, and May 4, 1943. During this period we saw a total of 221 bacteriologically proved cases

of meningococcic meningitis. Eighty of these had been treated with a sulfonamide other than sulfadiazine, by a combination of sulfonamides, or by a combination of sulfonamide and serum. These cases have not been included in this series. The remaining 141 cases, which were treated with sulfadiazine alone, constitute a consecutive series for the use of this drug and form the subject of this report.

Most of these patients were seen by us shortly after the onset or relatively early in the course of the disease. In many instances, however, we were first consulted after the patient had developed a complication or had apparently failed to respond to the chemotherapy.

**Clinical Aspects.** Table 1 shows the age distribution of the patients in this series. For the purpose of comparison we have also included in this table the age distribution of the 80 cases treated with other sulfonamides or sulfonamide and serum.

TABLE 1.—THE AGE DISTRIBUTION OF 141 CASES OF MENINGOCOCCUS MENINGITIS TREATED WITH SULFADIAZINE ALONE AND OF 80 CASES TREATED WITH OTHER SULFONAMIDES OR WITH SULFONAMIDES AND SERUM

Age (in years)	Cases treated with sulfadiazine alone	Cases treated with other sulfonamides or with sulfonamide and serum
Under 1	6	1
1-10	37	6
11-20	40	22
21-30	20	24
31-40	21	11
41-50	8	11
51-60	6	4
61-70	3	1
	141 cases	80 cases

The duration of illness before treatment with the drug varied from 1 to 8 days. As shown in Table 2, 126 of the 141 patients were treated within the first 3 days of illness.

TABLE 2.—DURATION OF ILLNESS BEFORE CHEMOTHERAPY

Days before chemotherapy	Number of cases
1	69
2	38
3	18
4	9
5	4
6	2
7	0
8	1

Most of the patients presented the typical picture of meningitis on admission, including nuchal rigidity and positive Brudzinski and Kernig signs. Twenty-six of the patients were mentally clear and 115 showed definite changes in the mental state, varying from irritability and apathy to delirium and coma. A hemorrhagic eruption was present in 92 cases. Arthralgia or arthritis was observed on admission in 9 instances. The individual patients showed considerable variation in the severity of the disease, but most of them appeared critically ill on admission and some even moribund.

The 8 cases of meningococcus bacteremia without evidence of meningal involvement, showed the usual septic temperature and the presence of an extensive hemorrhagic or polymorphous eruption. Arthralgia was present in 4 instances. The spinal fluids were essentially normal.

**Bacteriologic Diagnosis.** Diagnostic lumbar punctures were performed on all of the cases of meningitis on admission. The cerebrospinal fluid showed varying degrees of turbidity. The cells were greatly increased in number with polymorphonuclears predominating. In a majority of instances the protein content was moderately to greatly increased. The sugar was either absent or markedly diminished in almost all instances, although in a few cases seen early in the disease the sugar content was within the normal range.

The diagnosis was confirmed bacteriologically in all cases. Stained smears of the cerebrospinal fluid showed varying numbers of intracellular and extracellular Gram-negative diplococci in 123 instances. Meningococci were grown from the fluid in 110 of the 131 cases in which cultures were made. In 95 cases the organisms were found both by smear and culture. In 3 instances with spinal fluid findings indicative of purulent meningitis but absence of organisms by smear and culture, bacteriologic diagnosis was confirmed by recovering meningococci from the blood cultures. Blood cultures were made in 85 of the cases of meningitis and were positive in 32 instances. The blood culture was positive in the 8 instances of meningococcemia without evidence of meningitis.

The strain of meningococcus was identified in 99 of the cases of meningitis and in the 8 cases of meningococcemia without meningial involvement. The organisms were typed directly from the spinal fluid by the Quellung method in 28 instances. In 20 cases, the meningococci obtained from the spinal fluid cultures were agglutinated by type specific sera. In the remaining 59 instances, the type was determined by agglutinating known strains of meningococci with the serum of the patient. Of all these strains, 103 were Type I, 1 was Type II, and 3 were Type IIA.

**Method of Treatment and Dosage.** All the patients were treated with sulfadiazine, its sodium compound, or a combination of both. With the exception of 1 of the fatal cases (Case I below) in which, after a thorough trial of sulfadiazine, 11 gm. of sodium sulfapyridine were administered shortly before death, none of the patients received any other sulfonamide drug and none received serum. While not all of the patients were treated in a strictly uniform manner, the method of therapy that we have adopted was employed in a majority of instances. In general, we have used the following scheme of dosage: On the first day of treatment, children under 1 year of age received from 2 to 3 gm.; children from 1 to 3 years of age received from 3 to 4 gm.; children above that age received from 4 to 7 gm.; and adults from 9 to 12 gm. One-quarter to one-third of this amount was given as the initial dose and the remainder divided and administered at intervals of 4 hours. In 89 instances the drug was given by vein during the first 12 to 24 hours, as a rule in a 5% solution in distilled water. In very severe cases the parenteral medication was continued for a longer period of time. In the remaining cases the drug was administered orally from the start. It is our belief that the parenteral route should be reserved for the severe cases and for those patients who are unable to take or retain oral medication. There is no indication for the use of the drug intrathecally. In our opinion it is dangerous to make the first dose a massive one, especially when given by vein. Similarly, it is undesirable to give large amounts of the drug simultaneously by vein and by mouth. Following the first 12 to 24 hours, the patient is as a rule able to take medication orally, and he continues to receive the drug at 4 hour intervals.

In the use of the drug after the 1st day, we are guided by the clinical condition of the patient and the concentration of the sulfadiazine in the blood. It is essential that during the course of sulfonamide therapy the patient should receive a sufficient amount of fluid to maintain an adequate renal output, particularly when large amounts of the drug are administered intravenously. We agree with Downing and Lepper<sup>8</sup> in recommending the administration of at least 3000 cc. of fluids a day in order to insure a urinary output of 1200 cc. or more daily. This is of particular importance in cases of meningitis because of the danger of dehydration from vomiting early in the course of the disease.

**Concentration of Sulfadiazine in the Blood and Cerebrospinal Fluid.** We wish to stress the importance of making frequent determinations of the sulfadiazine level in the blood, since its concentration in the blood and cerebrospinal fluid of a given patient is not definitely predictable on the basis of dosage. While there is no conclusive evidence of a definite correlation between the effectiveness of sulfadiazine and its concentration in the blood and cerebrospinal fluid, nevertheless we have obtained prompt and consistent results with drug levels ranging from 10 to 15 mg. per 100 cc. in the blood and from 7 to 10 mg. per 100 cc. in the cerebrospinal fluid. Excessive concentration may lead to drug intoxication. Although some patients can tolerate high concentrations of the drug without untoward effects, the maintenance of a high sulfonamide level is distinctly hazardous. While emphasizing the importance of optimal drug levels, we recognize that some patients may show a satisfactory clinical response to relatively low concentrations. In such instances it is, of course, unnecessary to increase the amount of sulfadiazine.

Long<sup>16</sup> and Finland and his collaborators<sup>17</sup> have pointed out that sulfadiazine readily enters the cerebrospinal fluid and maintains a level of about two-thirds to four-fifths of that found in the blood. Determinations made on comparative blood and cerebrospinal fluid levels in 14 instances would seem to support these observations, as shown in Table 3. In joint exudates the concentration of the drug more nearly approximates that of the blood.

TABLE 3.—THE COMPARATIVE CONCENTRATION OF SULFADIAZINE IN THE BLOOD AND CEREBROSPINAL FLUID

Concentration in mg. per 100 cc.	
In blood	In spinal fluid
6.8	5.5
7.6	5.3
9.0	5.0
9.3	8.8
11.3	8.7
12.0	12.2
14.0	8.0
16.0	7.5
19.8	9.5
23.0	10.2
28.7	15.8
31.0	15.0
	24.0
	17.0

Average =  $\frac{15.1}{10.9}$   
 The concentration in the spinal fluid averages 72% of that in the blood.

**Results.** The rapidity with which the serious symptoms disappeared in most of the cases was little short of astounding. As a rule, the patients showed striking clinical improvement on the 2nd or 3rd day of chemotherapy. In most instances the fever dropped by crisis (Fig. 1) or by rapid lysis. Occasionally the lysis was slower. While subjective improvement was apparent early, the abnormal neurologic signs usually persisted for a longer period. Indeed, nuchal rigidity not infrequently lasted well into convalescence.

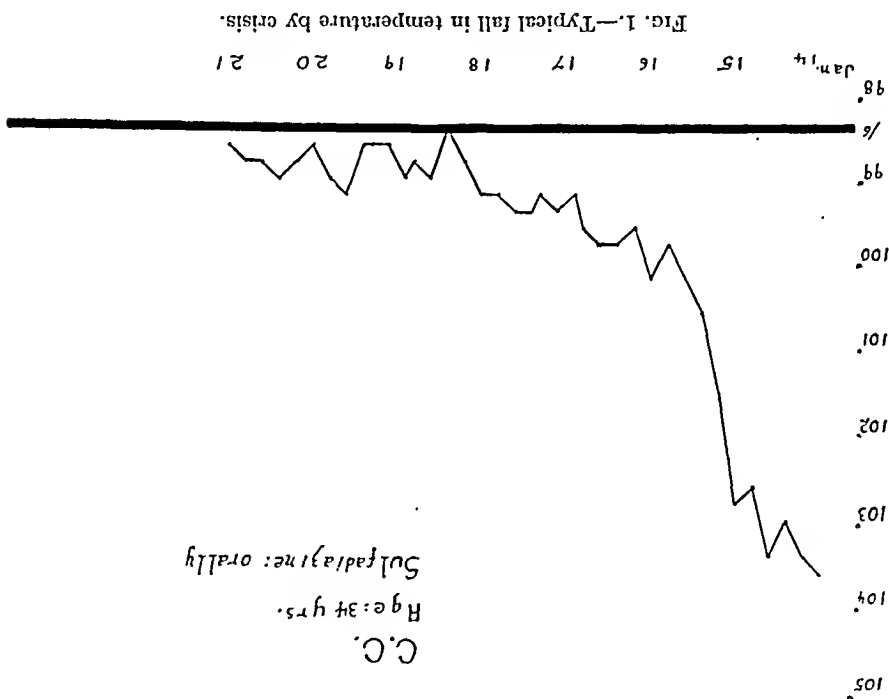


FIG. 1.—Typical fall in temperature by crisis.

Following the initial response, there was usually progressive clinical improvement and the temperature remained normal. Subsequent elevations in temperature were as a rule indicative of either a drug intoxication or the development of a complication. These features will be discussed in detail in another section of the paper. There is at present a great deal of uncertainty with regard to the duration of the chemotherapy and the total amount of drug required in each case. Early in our study, the administration of sulfadiazine was continued for about 4 to 5 days after the temperature had become normal. More recently, we have found that one may safely discontinue chemotherapy after the patient has been afebrile for 24 to 48 hours and has received a total of 15 to 20 gm. of the drug. We do not think it is necessary to use more than that amount of sulfadiazine in the average case, although an occasional instance may require a larger quantity. As a matter of fact, we have seen recovery with 12 gm. or less in 16 instances, in 4 of which the patient received a total of only 7 to 8 gm. of the drug. It is unnecessary and may even prove harmful to administer too much of the sulfonamide or to use it too



long. The ill-effects from overtreatment are, in our experience, much more frequent than is generally realized.

The question naturally arises as to when the infection may be regarded as having come under control. We have found that, with few exceptions, the disease is well controlled when the patient shows a decided improvement in the mental state, has been essentially afebrile for 24 to 48 hours, and has received a total of 15 to 20 gm. of the drug. As a rule, it is unnecessary to resort to lumbar puncture for the purpose of evaluating the progress of the case. An examination of the cerebrospinal fluid may be indicated in those instances in which the course of the disease is unclear or the cause of a persistent fever is not apparent.

In this connection it may be of value to comment on certain features of the spinal fluid examination. Undue stress is often laid on the importance of the total cell count. We have often discontinued the chemotherapy in the presence of a considerable pleocytosis. A rise in the sugar content of the cerebrospinal fluid and the disappearance of the organisms are the most important determinations. However, control of the infection is not infrequently attained before the return of the sugar to normal. Primary reliance on symptomatic improvement as a guide to drug dosage has not resulted in any relapse or recurrence of the infection in the cases in this series or in any of our cases treated with other sulfonamides.

We have already mentioned that in this series of 141 cases of meningococic meningitis there were 139 recoveries and 2 deaths, a mortality of 1.4%. The 8 patients with meningococemia but without meningeal involvement recovered promptly and completely. It may be noted that if to the series of 141 cases treated with sulfadiazine, we add the group of 80 cases treated with other sulfonamides or with a combination of sulfonamides and serum, we obtain an over-all mortality rate of 3.6%.

It may be of value to comment briefly on the 2 fatal cases of meningitis in the sulfadiazine-treated series.

**Case Studies.** Case 1. J. V., male, aged 43, was admitted to the hospital on the 2nd day of illness with signs of semistupor and meningeal irritation. The temperature on entry was 100.6° F. and rose rapidly to 102.4° F. The diagnosis was established by spinal fluid findings which showed meningococci by smear and culture. In the first 12 hours a total of 14 gm. of sulfadiazine was administered by vein resulting in a drug level in the blood of 27 mg. per 100 cc. after 21 hours. The temperature dropped to 98.6° F. For some unexplained reason the patient went into a state of shock from which he recovered following a blood transfusion. Sulfadiazine which had been temporarily withdrawn was resumed by mouth on the 4th hospital day and was continued for 5 days during which 26 gm. were administered. The fever persisted, ranging at first from 100° to 101° F. and rising on the 7th day to 104° F. A partial clearing of the mental state during the 6th and 7th hospital days was only temporary and was followed by a progressive deterioration of the patient's general condition. Sodium sulfapyridine (11 gm.) was given intravenously but also without avail. Decubitus ulcers and cellulitis of the left foot developed terminally. Death occurred on the 10th hospital day. A necropsy was not obtained.

The cause of the circulatory collapse in this patient was obscure. It is

obvious that the chemotherapy was too intensive at the start and was probably continued too long. However, this case must be recorded as an instance of failure to respond to sulfadiazine.

CASE 2. M. F., a 40 year old female, suddenly became ill on March 19, 1943, with headache, vomiting, fever and severe chills. The following day she was admitted to the hospital in coma and in a state of circulatory collapse. The temperature was 101.2° F., subsequently rising to 105.6° F. The heart rate was 160 but the peripheral pulse was not obtainable. The systolic blood pressure was 40 and the diastolic was unobtainable. The lungs showed evidence of pulmonary edema. There was a profuse hemorrhagic eruption and there were also subconjunctival and retinal hemorrhages. The neck was rigid and was associated with positive Brudzinski and Kernig signs. There was the suggestion of a right hemiplegia. The deep and superficial reflexes were absent. The cerebrospinal fluid was cloudy, showed 30,000 cells, predominantly polymorphonuclears, a sugar of 10 mg. per 100 cc. and meningococci on smear and culture. The blood culture was positive. The patient was treated with sodium sulfadiazine by vein, receiving in divided doses a total of 9 gm. However, she remained in coma and in a state of collapse and died 20 hours after admission.

This case obviously belongs to the fulminating type. It is to be noted that no treatment for shock was administered.

In this connection we would like to point out that our series included 3 other cases of the fulminating form, all of which fortunately recovered. These patients were admitted to the hospital in stupor and in a state of shock. Their blood pressures were either low or unobtainable. In addition, they showed varying degrees of meningeal irritation and a profuse hemorrhagic eruption. Meningococci were recovered either from the spinal fluid or from the blood. The patients were treated with sodium sulfadiazine by vein and concurrently for shock with desoxycorticosterone or cortical extract together with plasma or whole blood. Recovery was uneventful in all 3 instances. We do not wish to convey the impression that the outcome in the 4 fulminating cases cited by us represents the recovery rate in this form of the disease, since a number of patients died before they were reported to us or before they could receive appropriate treatment.

It is well known that in the fulminating form of the disease, aside from the extensive hemorrhagic eruption, the outstanding clinical feature is the presence of circulatory collapse. These patients have an overwhelming bacteremia and often show only a minimal degree of meningeal involvement. The most conspicuous necropsy finding is the presence of massive hemorrhages into the adrenal glands. While at the present time the fulminating cases are almost always fatal, it may be hoped that prompt and vigorous therapy for the circulatory collapse, in addition to chemotherapy, may bring about recovery in those instances in which the adrenals are only partly involved by hemorrhage.

**Complications and Their Therapy.** There were relatively few complications in this series. Arthritis involving one or more joints was the most common of these. It occurred in 15 cases. In 5 instances it was present on admission and in the remaining 10 cases it appeared anywhere from the 3rd to the 8th day. Most of the involved joints showed swelling and tenderness. A suppurative arthritis was relatively uncommon and was as a rule nonarticular. The development

of arthritis was an important cause of either a persistent or rising temperature after the 3rd day. The treatment of this complication consisted of immobilization of the involved joint, resumption of the sulfadiazine and, occasionally, aspiration. All of these patients re-

covered completely. One patient had a bilateral otitis media on admission. Following myringotomy, the condition cleared up completely. Cystitis was observed in 1 case. One patient developed an extensive left femoral thrombophlebitis on the 11th day. The condition responded promptly to sulfadiazine. There was one instance of uveitis, which was treated successfully with chemotherapy. It may be noted that before the advent of sulfonamide therapy this complication usually led to a panophthalmitis and permanent blindness. The low incidence of serious complications was quite remarkable.

**Toxic Effects.** Toxic reactions referable to sulfadiazine were encountered in 42 of the patients, 10 of whom had more than one untoward effect.

The most common toxic reactions encountered in this series were related to the urinary tract. Hematuria was observed in 20 patients, in 15 of whom it was microscopic and in 5, gross. Since we have no records of daily urine examinations in every case, it is possible that the incidence of hematuria was somewhat higher. Five of these were accompanied by oliguria or anuria. There were 3 instances of oliguria in which there was no record of a preceding hematuria. As may be seen from Table 4, most of the patients developed a renal complication on the 2nd to 4th day of chemotherapy. In 1 instance hematuria appeared 5 days after the withdrawal of chemotherapy. It may be noted also that the occurrence of these untoward reactions was not definitely attributable either to the total amount of drug used or to its level in the blood. Keitzer and Campbell<sup>13</sup> and Dowling and Leeper<sup>8</sup> believe that a diminished urinary output is probably an important factor in the causation of the renal complications. Although we do not have precise information on fluid balance in our series, it is our impression that this opinion is warranted. It is of importance, therefore, to maintain a urinary output of at least 1200 cc. in each 24 hour period.

The nature of the renal damage is at present not entirely clear. Several observers<sup>1,5,21</sup> have pointed out that there are 2 types of renal lesions, namely a mechanical blockage of the urinary passages by concretions of crystals, and toxic effects on the tubular epithelium. Leutcher and Blackman<sup>15</sup> have recently reported the finding of glomerular damage in 2 instances of sulfonamide intoxication.

Most of our renal reactions cleared up rapidly following the early institution of conservative measures. These included the immediate withdrawal of the drug, forcing of fluid and the application of heat to the back and the abdomen. In 2 instances, obstruction of the urinary tract resulting from the deposition of sulfadiazine crystals was relieved promptly by ureteral catheterization and pelvic lavage. In most of our cases the hematuria cleared up within 48 hours after the drug was

stopped. The finding of even a few red blood cells in the urine may be regarded as a danger signal and as an indication for the withdrawal of the sulfadiazine. This is a very important precautionary measure and indicates the need for daily urine examinations. It is safe to resume the chemotherapy, if necessary, 12 to 24 hours after the urine has cleared, as was demonstrated in 4 of our cases. There is growing clinical evidence<sup>11,12</sup> that alkalization of the urine increases the solubility of the drug and decreases the likelihood of renal reactions, particularly of the obstructive type. However, the presence of crystals in the voided urine should not be considered an indication for stopping chemotherapy provided the urinary output is good.

TABLE 4.—RENAL REACTIONS FOLLOWING SULFADIAZINE THERAPY

Case	Amount of drug (gm.)	No. of days drug was given	Chemotherapy prior to reaction		Nature of reaction	Clearing of urine (days)	Remarks
			Blood	Urine			
1	69	10	10		Hematuria-gross	2	Chemotherapy resumed. Urine remained clear.
2	17	2	10		Hematuria-micro	2	Chemotherapy resumed. Urine remained clear.
3	18	3	17		Hematuria-micro	3	Microscopic hematuria, albuminuria, edema. Felt lavage.
4	20	2	17		Hematuria-micro	3	Microscopic hematuria, albuminuria, edema. Felt lavage.
5	12	1	11.1		Oliguria with micro-hematuria	4	Azotemia. Uteral catheterization. Impaction washed out.
6	19	4	..		Hematuria-micro	3	Chemotherapy continued for 2 days more. When discontinued, urine cleared.
7	22	2	8.3		Hematuria-micro	2	Arthritis. Chemotherapy resumed. Urine remained clear.
8	30	5	14		Hematuria-micro	1	Urine remained clear.
9	7	1	11.2		Hematuria-gross	1	
10	30	3	..		Hematuria-micro	..	
11	22	4	..		Hematuria-micro	2	Renal pain.
12	16	2	..		Hematuria-gross	2	Renal pain.
13	3	1	21		Oliguria with micro-hematuria	..	Began after 3 gm. of sulfadiazine by vein. Chemotherapy continued.
14	10	2	11		Hematuria-micro	2	Urine cleared.
15	17	2	7.6		Hematuria-micro	4	Hematuria appeared 4 days after chemotherapy discontinued.
16	14	2	..		Hematuria-micro	..	Increased fluid intake. Oliguria cleared. Arthritis. Chemotherapy continued.
17	14	2	11.2		Oliguria	2	Edema. Improvement rapid.
18	10	2	..		Oliguria with hematuria-gross	2	
19	12	2	50.2		Oliguria	..	
20	6	1	41.4		Hematuria-gross	1	
21	12	2	9.1		Oliguria	..	No hematuria.
22	9	1	..		Oliguria with hematuria-micro	..	Followed by oliguria and edema with transient hypertension as chemotherapy was continued and level rose to 46 mg. %.
23	6	1	14.7		Hematuria-micro	1	Chemotherapy then stopped; urine cleared. Began 7 hours after chemotherapy started.

The second most important toxic effect that we have encountered in our series was drug fever, which was noted 15 times. In 8 patients it occurred alone and in 7, in conjunction with some other form of intoxication. When the drug fever appeared alone, its onset was as a rule early in course of treatment. It is probable that this complication

was even more frequent than is indicated by our figures, since its recognition is often difficult. Contrary to the general belief that this reaction occurs toward the end of the 1st week, we have usually noted its appearance as early as 24 or 48 hours after the beginning of chemotherapy. This early drug fever was suspected when, after the initial defervescence, followed by a short period of clinical improvement varying from a few hours to 1 or 2 days, the patient became subjectively worse and the temperature rose (Fig. 2). The ascent of the fever was usually step-like or irregular in manner. In all but 1 of the cases the fever persisted as long as the chemotherapy was continued. Withdrawal of the drug resulted in a fall of the temperature by crisis in all but 1 instance. In this exceptional case the defervescence was by lysis lasting 4 to 5 days. There was 1 instance in which the fever developed on the day the drug was discontinued and, following a course of 2 days duration, dropped by crisis.

When it is difficult to decide whether the secondary rise in temperature is the result of drug intoxication or the persistence of the infection, spinal fluid findings show evidence of improvement such as a drop in the cell count, an increase in the sugar and an absence of organisms, one may safely withdraw the drug. On the other hand, if the spinal fluid findings indicate a still active infection it is necessary to continue the chemotherapy.

Although there is a growing body of evidence indicating that all

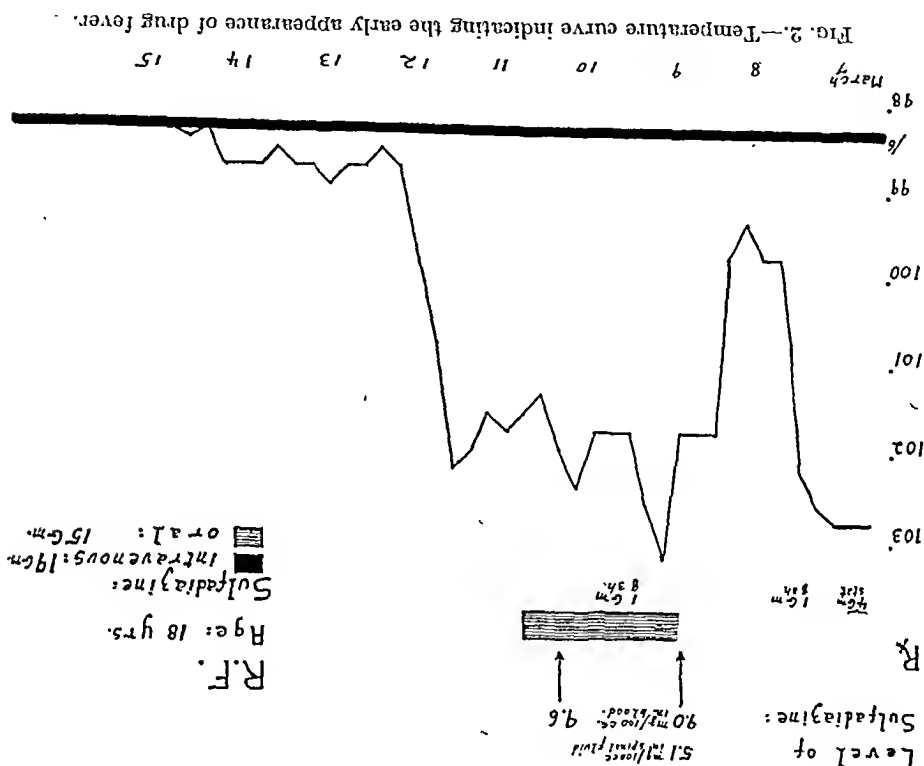
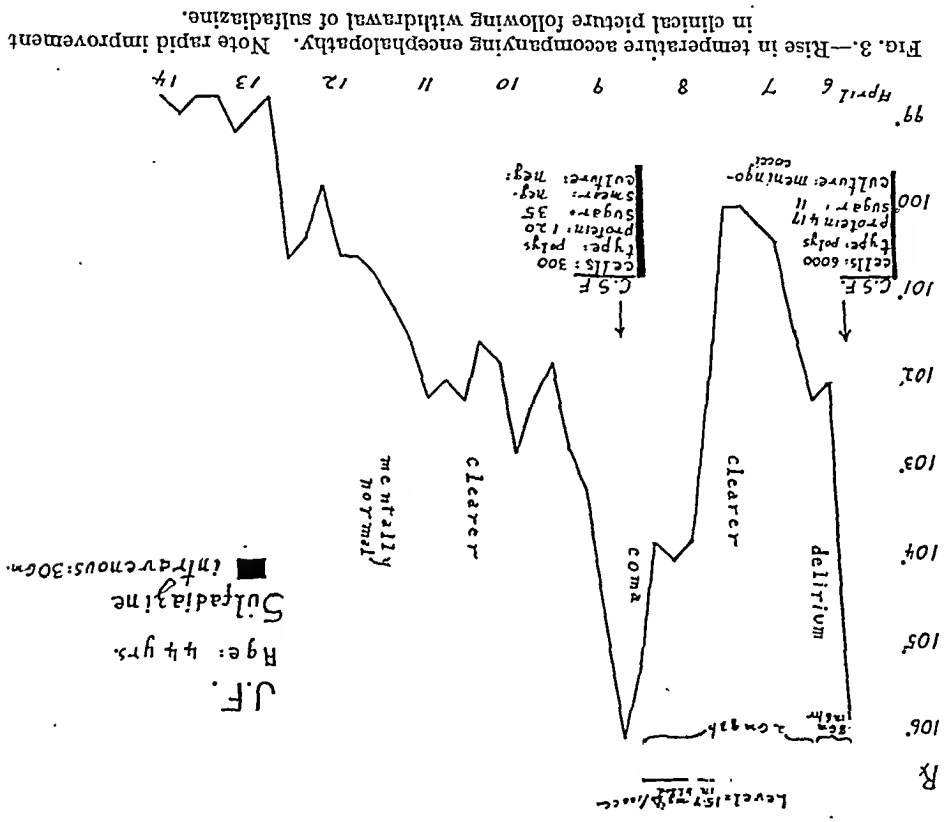


TABLE 5.—ENCEPHALOPATHY DUE TO SULFADIAZINE INTOXICATION

Chemotherapy prior to onset  
of complication

Case	Age (yrs.)	Symptoms of encephalopathy	No. of days drug given	Amount of drug (gm.)	Route	Level of drug in blood (mg. per 100 cc.)	Spinal fluid findings		Course and outcome
							On entry	During encephalopathy	
1	37	Mounting fever; delirium with hallucinations	2	20	10 gm. intrav. 10 gm. oral	26.4	Cells: 26,000 (polys); protein: 4+; sugar: absent; smear: men- ingococci; culture: meningococci	Cells: 2300 (lymphos); protein: 4+; sugar: 3+; smear: neg.; culture: neg.	Drug cont. 6 days; symptoms persisted, with mount- ing fever and clearing spinal fluid. When drug was stopped temp. dropped sharply from 105° F. to normal with prompt clin. improvement.
2	1	Moderate fever, convulsions, coma	3	11	4 gm. intrav. 7 gm. oral	31.0	Cells: 3150 (polys); protein: 2+; cul- ture: meningococci	Cells: 2500 (polys); protein: 36 mg./100 cc.; sugar: 80 mg.; no organisms	Drug cont. 9 days. Remained in semi-stupor and developed blindness and deafness. When drug was stopped, gradual improvement. Later return of vision and hearing; persistence of convulsions.
3	35	Mounting fever, coma	2	17	All by vein	38.0	Cells: 60,000 (polys); protein: 840 mg.; sugar: ab.; smear: meningococci; cul- ture: meningococci	Cells: 500 (polys); sugar: 50 mg.; no or- ganisms by smear or culture	Drug cont. 7 days with mounting fever and coma. When drug was stopped, temperature dropped by lysis and mental state cleared.
4	44	Rapid rise in temperature, deep coma	2	30	All by vein	15.7	Cells: 6000 (polys); protein: 417 mg.; sugar: 11 mg.; cul- ture: meningococci	Cells: 300 (polys); pro- tein: 120 mg.; sugar: 35 mg.; no organisms by smear or culture	Drug stopped 3d day <sup>1</sup> when temperature was 103° F. Immediate improvement in mental state and temperature dropped to normal in 4 days.

members of the sulfonamide group have a toxic effect on the nervous system, the subject has not received sufficient attention. A number of investigators<sup>3,7,17</sup> have noted the development of injury to the nervous system of animals fed with a variety of sulfonamides. Clinical reports<sup>2,4,6,10,14,18,19,20</sup> indicate that these drugs produce widely varied types of toxic nervous reactions. These include the peripheral neuropathies, deafness, optic neuritis, blindness and other visual disturbances, encephalomyelitis, encephalopathy with or without convulsions, mental confusion, psychoses, and disturbances of speech. Little<sup>14</sup> has emphasized that sulfonamides appear more neurotoxic in the presence of preëxisting disease of the nervous system.



We have encountered 4 cases in which drug intoxication produced the syndrome of encephalopathy. This complication is usually difficult to recognize and to differentiate from the symptomatology of the meningitis. Table 5 shows the important clinical features. The onset of this condition was early in the course of treatment, usually appearing on the 2nd or 3rd day. The clinical picture was characterized mainly by varying grades of stupor often progressing to deep coma. Occasionally there were delirium, hallucinations, or convulsions. These symptoms were invariably accompanied by a significant rise in temperature (Fig. 3). It may be noted that the patient with convulsions developed also bilateral blindness without fundus changes together

Fig. 3.—Rise in temperature accompanying encephalopathy. Note rapid improvement in clinical picture following withdrawal of sulfadiazine.

with bilateral deafness, both of which cleared up late in convalescence. It is our impression that these latter complications were due to drug intoxication, since the blindness and deafness caused by the infection are practically always permanent. One patient developed euphoria and garrulousness during the course of chemotherapy. In 1 case without evidence of encephalopathy there was a transitory unilateral tinnitus, apparently as a result of drug intoxication. It is important to note that in all of the cases of encephalopathy examination of the spinal fluid during the development of the intoxication showed definite evidence of improvement. This fact was of help in ruling out the infection as the cause of the aggravation of the clinical picture. In 1 of the patients, withdrawal of the drug resulted in a precipitous drop in the temperature and an immediate improvement in the mental state. The other 3 patients improved more gradually after cessation of chemotherapy.

There were 2 instances of peripheral neuropathy in this series. In 1 case the infiltration of the soft tissues of the antecubital space during intravenous chemotherapy resulted in a paralysis of the radial nerve. One month after the development of the complication, the wrist drop still persisted. It is difficult to say whether the nerve injury was caused by mechanical pressure or by a local toxic effect. The other case was one of meningococemia without evidence of meningitis but with a polyarthrits. After a total of 10 gm. of chemotherapy a left foot drop developed on the 3rd hospital day. The drug was discontinued but was resumed on the 7th hospital day because of the progression of the arthritis. Two days following the resumption of chemotherapy during which 9 additional grams were administered the patient developed a right wrist drop. There was considerable improvement of the neuropathy within 2 weeks. In contradistinction to the first case, it is quite evident that in this instance, the neurotoxic effect of the drug was systemic in character.

A variety of blood dyscrasias, including agranulocytosis thrombocytopenic purpura and hemolytic anemia have been reported following sulfadiazine treatment. We have not encountered any of these serious blood reactions. Moderate drops in the leukocyte count from high or normal figures to below normal occurred in 3 cases (from 22,000 to 4320; 20,950 to 4800; 9000 to 4800). These did not constitute an indication for withdrawal of the drug. There were 3 patients with initial leukocyte counts below 6000 whose counts rose during therapy. In 1 of these, the number of leukocytes rose from 1100 before treatment to 12,000 on the 2nd day of treatment. It is evident that the presence of an initial leukopenia is not a contraindication to the use of chemotherapy.

A skin eruption was noted in 3 patients. This was of the morbilliform type and was associated in each instance with a slight rise in temperature ranging from 100° to 101.8° F. In 1 case the rash appeared on the 2nd day of therapy, and in the other 2, on the 4th day. The total amounts of the drug received prior to the development of the cutaneous eruption were 11, 16 and 30 gm. respectively. The concen-



tation of the drug in the blood was relatively low in all of the cases (5.3, 7.5 and 8 mg. per 100 cc.). It would seem that the development of this complication is not dependent on either the total amount of the drug administered or its concentration in the blood. It should be noted that in each instance the rash disappeared within 24 to 48 hours after the withdrawal of the drug.

We have discussed the toxic reactions at length because a knowledge of these is of basic therapeutic importance. The intelligent planning of dosage and the proper evaluation of the patient's response to chemotherapy are in large measure dependent upon the physician's understanding of the untoward effects. Constant vigilance and the prompt institution of remedial measures upon the earliest appearance of toxic signs will insure the safe use of this very valuable drug.

**Failure of Response.** We were consulted in 35 cases reported to us as chemotherapeutic failures. Twenty-two of these proved to be instances of some form of drug intoxication. In 11 cases the development of arthritis misled the physician into the belief that the patient was failing to respond to the chemotherapy. The 2 remaining cases were the fatalities described above. The presence of an associated condition such as cardiovascular disease, nephritis, or tuberculosis may interfere with the success of sulfonamides. We are convinced that, excepting those cases which are of the fulminating form or which have a severe associated disease, no patient with meningococcus meningitis or meningococcemia should fail to respond to properly administered chemotherapy.

There is evidence, both experimental and clinical, that under certain circumstances bacteria develop a resistance to sulfonamides. Most of the studies on sulfonamide resistance have been obtained for the pneumococcus. We have not encountered any case of meningococcal meningitis or meningococcemia in which drug fastness appeared to be the cause of failure of response.

In several cases reported to us as sulfonamide failures, we were able by careful and repeated study of the spinal fluid to prove an error in diagnosis. The causative organisms, originally recorded as meningococci, proved to be *H. influenzae* or Gram-positive cocci which had been overdecolorized during staining. As is well known, meningitis caused by these organisms does not respond to chemotherapy as satisfactorily as the meningococcal form.

**Remarks on Serum.** In view of the excellent results obtained with sulfadiazine in this series and with other sulfonamides in a large number of cases not included in this study, it is difficult to see what contribution one can expect from the additional use of the specific serum. In cases of apparent failure of response to sulfonamide therapy the use of the specific horse serum has not in our experience produced any striking benefit. We have already pointed out that when sulfonamide therapy does not result in prompt improvement it is necessary to determine and correct the underlying cause of the apparent failure.

**Summary and Conclusions.** 1. Sulfadiazine and its sodium compound were used in the treatment of 141 bacteriologically proved cases

of meningococcic meningitis and 8 cases of meningococcemia without meningeal involvement.

2. Our method of treatment and dosage have been discussed in detail. We have found that with few exceptions the disease was well controlled and chemotherapy could be safely discontinued when the patient shows a decided improvement in the mental state, has been essentially afebrile for 24 to 48 hours and has received a total of 15 to 20 gm. of the drug.

3. The rapidity with which most of the serious symptoms disappeared was little short of astounding. Of the 141 patients with meningitis, 139 recovered and 2 died, a mortality of 1.4%. The 8 patients with meningococcemia but without meningeal involvement also recovered.

4. There were relatively few complications in this series. The most common of these was arthritis, which occurred in 15 cases.

5. Toxic reactions were encountered in 42 of the patients, 10 of whom had more than one untoward effect. The most common of these toxic reactions were related to the urinary tract. The second most important toxic effect was drug fever, which usually occurred as early as 24 to 48 hours after the institution of chemotherapy. We have encountered 4 cases of encephalopathy resulting from sulfadiazine intoxication in this series. There were also 2 instances of peripheral neuropathy following sulfadiazine intoxication. A morbilliform skin eruption was noted in 3 cases and was associated in each instance with a slight rise in temperature. With the exception of the peripheral neuropathies all the toxic reactions cleared up rapidly following withdrawal of the drug and the institution of remedial measures.

6. In cases of apparent failure of response to sulfonamide therapy the use of the specific horse serum has not, in our experience, produced any striking effect. Most of the cases reported to us as chemotherapeutic failures were instances of intoxication, often the result of overtreatment. We are convinced that, excepting those cases which are of the fulminating form or which have a severe associated disease, no patient with meningococcic meningitis or meningococcemia should fail to respond to properly administered chemotherapy.

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## THE CHIEF URINARY PIGMENT

### THE RELATIONSHIP BETWEEN THE RATE OF EXCRETION OF THE YELLOW URINARY PIGMENT AND THE METABOLIC RATE

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The yellow color of the urine is at once a striking yet poorly understood characteristic.

Some years ago Drabkin<sup>1</sup> described a method for measuring pigment concentration by comparison with a standard dye solution in a colorimeter, and has studied the pigment output in relation to basal metabolic rate. He concluded that the pigment is constant from day to day and that in several species, the pigment output was roughly proportional to the surface area and the urinary pigment output was proportional to the basal metabolic rate in hyperthyroid patients. Drabkin inferred that the urinary pigment was a product of endogenous metabolism, and that the rate of elimination of this pigment was proportional to the intensity of metabolism. Confirmation of this thesis was given in a second paper, among hyperthyroid patients, in some cases both before and after thyroidectomy, and among febrile patients, basal metabolic rates could be correlated with the daily output of urinary pigment. Drabkin<sup>2</sup> did not believe, however, that this correlation was close enough to justify calculation of the metabolic rate from measurement of the urinary pigment.

The present authors have reinvestigated the nature of the relationship between urinary pigment output and the rate of metabolism using other procedures and controls than those employed by Drabkin.

**Methods.** Our technique for the estimation of the urinary pigment was a purely photometric one. In our earlier work, we used the Pulfrich Stufenphotometer, comparing light intensities through the S43 filter (peak wavelength 430 millimicrons). This, among 7 filters distributed throughout the spectrum, gave results which were most consistent and most closely related to

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BMR. Latterly, the Klett photoelectric colorimeter was employed, the light passing through the Klett standard blue filter. Both the negative logarithm of the percentage of light transmitted through the solution as given by the Pulfrich instrument and the dial reading given by the Klett instrument were found to be proportional to the relative concentration in samples made by careful dilution of a single urine specimen, thus confirming the fact that Beer's law obtains for our readings. Although we realized that we had not ruled out the possibility of the existence of 2 or more urinary pigments which absorbed blue light, for the purposes of this investigation we defined the yellow urinary pigment as that pigment whose concentration was measured by the negative logarithm of the percentage of blue light (peak 420-430, relatively monochromatic) transmitted through a standard length of urine. For our purposes, this negative logarithm was treated as the concentration itself and the product, the negative logarithm by the volume as the amount of pigment.

The creatinine content of the urine was determined by one of two variations of the Jaffe reaction.<sup>4</sup> In each a mixture of 10% sodium hydroxide, saturated picric acid and urine was made up in the following proportions: (1) 1:3:2 cc.; (2) 1.5:20:1 cc. After standing 10 minutes, the mixture was diluted to 100 cc. and the color intensity compared with that yielded by a standard solution of creatinine (1 mg. per cc.) using the visual colorimeter in the first case and the Klett photoelectric colorimeter with green filter in the second. When the second method was calibrated against known dilutions of the standard solution a smooth curve was obtained characterized by the equation:  $2y = x + x^2$ , where  $y$  represents the known concentration of the sample in mg. per cc. and  $x$  represents the apparent concentration. Whereas an error of  $\pm 15\%$  at a concentration of 0.5 mg. per cc. and  $-20\%$  at 2 mg. per cc. is implied, most of our results fell between 0.8 and 1.2 where the errors were less than  $\pm 6\%$ . These errors were eliminated by means of the calibration curve. No attempt was made to calibrate the first method. It should be pointed out that the proportions of sample and reagents in Method 1 are those originally prescribed by Rollin and are known to give correct readings for ratios varying from 2:1 to 1:2. The proportions in the second method were those in current use in the hospital laboratory and contained an excess of picrate, the yellow color of which forms a large part of the total color.

To prevent variations in the apparent concentration of urinary pigment due to chemical change after voiding and to manipulation, the following procedure was adopted: Urine specimens were placed in an icebox immediately after being voided and photometric readings were made as soon as possible, though never later than 2 hours thereafter. Immediately before examination, all specimens, whether or not they appeared clear, were subjected to 10 minutes of high speed centrifugation, which was prolonged, if necessary to clear the urine. Dilutions were made as necessary. The subjects for the intensive studies were male medical students and young physicians in good health. The subjects of the extensive studies were those patients from a general medical and surgical service for whom estimation of BMR was ordered by the physicians in charge and from whom specimens could be collected. In each case unusual physical activity was avoided on the day before collection of the specimen, and beets and rhubarb were excluded from the diet.

**Results.** After standing, urines were observed to show apparent increase in concentration of pigment. At room temperature this amounted to more than 10% within 2 hours and 16% within 18 hours; in the icebox the apparent increase was 2% within 2 hours and 6% within 18 hours. Alteration of urinary pH and specific gravity within physiologic limits revealed that no correction for these factors was indicated. Light absorption readings were diminished by at least 10% if the urine was filtered through a single thickness of filter paper.

Four subjects submitted 5 series of specimens at 1- to 3-day intervals. On most occasions 1 specimen of urine was collected upon arising in the morning, the bladder having been emptied last at bedtime, and another was collected after about an hour of rest in basal condition, the bladder having been emptied at the beginning of the hour. The hourly excretion of urinary pigment was calculated by multiplying the pigment concentration by the volume of urine presented and dividing the product by the length of the collection period in hours. The following is a typical series of data (Subject D) for rate of pigment output during overnight and basal periods, respectively ( $x$  = not obtained): 47-x, 53-x, 44-54, 49-x, 49-x, 44-x, 45-x, 46-57, 43-45, 48-48, 48-61, 50-64, 38-54, 47-52, 52-71, 60-80, 48-61 and 48-57. The results, including coefficients of variation (*i. e.*, standard deviation divided by the mean, expressed in per cent) are presented in Table 1.

TABLE 1.—CONSTANCY OF HOURLY PIGMENT OUTPUT

Coefficient of variation (%)		Standard deviation (units per hour)		Mean (units per hour)		No. of samples		A (Series 1)		B (Series 2)		C		D	
Over- night	Basal	Over- night	Basal	Over- night	Basal	Over- night	Basal	Over- night	Basal	Over- night	Basal	Over- night	Basal	Over- night	Basal
14.1	14.0	6.7	6.1	48.0	43.6	5	0	16	14	15	29	18	12	15.1	13.5
8.7	7.3	10.5	3.2	60.4	36.7	7	0	14	14	15	29	18	12	15.1	13.5
17.4	11.9	4.8	6.2	57.0	49.3	11	11	29	15	15	29	18	12	15.1	13.5
8.3	10.4	8.9	4.8	58.7	45.7	12	12	18	15	15	29	18	12	15.1	13.5

To correct for weight, height, habitus and age among the subjects, the rate of pigment excretion was compared to the rate of excretion of creatinine. The ratio pigment concentration/creatinine concentration for Subject A, Series 2 showed a coefficient of variation of 9.4, not significantly different from the coefficient for pigment alone, 8.7. For an afebrile 17 year old girl with a receding pleural effusion, presumably tuberculous, the following series of ratios was obtained during basal periods: 206, 175, 168, 185, 232, 225, 201, 187, 228; this corresponds to a coefficient of variation of 11.1. When 20 24-hour urine specimens donated by a class of male medical students were examined for pigment concentration and creatinine concentration, and ranks assigned, a positive correlation of 0.65 was obtained. In view of these results and because a ratio of concentrations eliminates the necessity of measuring urinary volumes per unit time, always a source of inaccuracy in the absence of catheterization; and because in the presence of thyroid pathology the numerator and denominator are expected to vary in opposite directions, thus giving wider excursions than either alone; the ratio P/C was examined as a possibly useful clinical datum. By the administration of thyroid extract to the point of toxicity, the basal metabolic rate was varied in the 4 subjects mentioned above, BMR determinations were made every 1 to 5 days, and on those days overnight and basal pigment determinations were made. Each series of data was examined to determine how closely the pigment values paralleled the BMR values, with respect to the trend of the

series, individual fluctuations and maintenance of proper proportions. Ten series of data were obtained; 5 dealing with specimens collected during overnight periods and 5 during basal periods. Of each 5, 2 included creatinine data. The results of a typical experiment are given in Table 2. Whereas the pigment excretion per hour in the basal state gave a slightly better correlation with the BMR, the P/C ratio was considered preferable for reasons given above.

TABLE 2.—RESULTS OF A TYPICAL EXPERIMENT

Date	BMR	Pigment per ½ min.	Creatinine per ½ min. (mg.)	P/C
3/9/42	1	..	..	111
3/11	9	..	..	109
3/13	3	..	..	110
3/16	1	..	..	131
3/18	6	..	..	96
3/20	9	..	..	120
3/23	11	..	..	129
3/27	19	..	..	136
4/1	24	..	..	108
4/3	27	99	0.62	160
4/6	7	76	0.61	124
4/8	20	66	0.53	125
4/10	14	82	0.60	137
4/15	1	70	0.65	108
4/17	14	64	0.65	98
4/20	9	65	0.64	101
4/22	1	70	0.61	114
4/24	2	66	0.72	92

NOTE.—First dose of thyroid extract taken on 3/11, last on 4/2. Urine collected immediately after BMR determination; sample collected one hour earlier discarded.

To test the reliability of single P/C values for the estimation of BMR, urine samples were collected from each of the adult service patients who were scheduled for BMR determination about 1 hour before and immediately after the procedure. P/C was determined for the second sample only. The Klett photoelectric photometer was used. The data for females (Fig. 1) were much more satisfactory than those for males; 63 determinations were included, representing 34 patients. BMR readings varied from 72% of normal to 155% with a mean of 111% and a standard deviation of 20.4%. P/C readings ranged from 143 to 545, averaging 263, with a standard deviation of 112.2. The correlation coefficient, assuming a straight line relationship, was 0.75 (Bernstein, 0.69).<sup>\*</sup> The standard error of estimate was 13.5%. The regression lines are  $BMR = 59.3 + 0.20 P/C$  ( $BMR = 53.9 + 0.22 P/C$ , Bernstein). Twenty-two observations were made on 15 male patients. Here, there was a much wider dispersion of readings than among female patients. In many of the cases which

<sup>\*</sup> Since Bernstein's linear correlation coefficient and regression are not well known, it may be *apropos* to remark here, that in this system, deviations are not squared, thus avoiding magnification of large errors. The regression lines computed by this method are those which fit the data best according to the method of least squares, if the weight of a deviation is taken inversely proportional to the absolute value of the deviation of the independent variable from its mean. "The method is strongly recommended for all cases in which the data lose reliability with increasing deviations from the mean . . . the influence of extreme doubtful readings is considerably lessened."

yielded the most aberrant results, clinical evidence supported the estimate given by P/C rather than the BMR determination itself. BMR values varied from 64 to 153%, averaging 118%, with a standard deviation of 24.8%. P/C readings ranged between 125 and 536 with a mean of 237 and a standard deviation of 112.2. The correlation coefficient of Pearson was only 0.30, while that of Bernstein in which wide variations are relatively devaluated, 0.51. The regression lines are respectively:  $BMR = 102.3 + 0.067 P/C$  and  $BMR = 96.7 + 0.090 P/C$ .

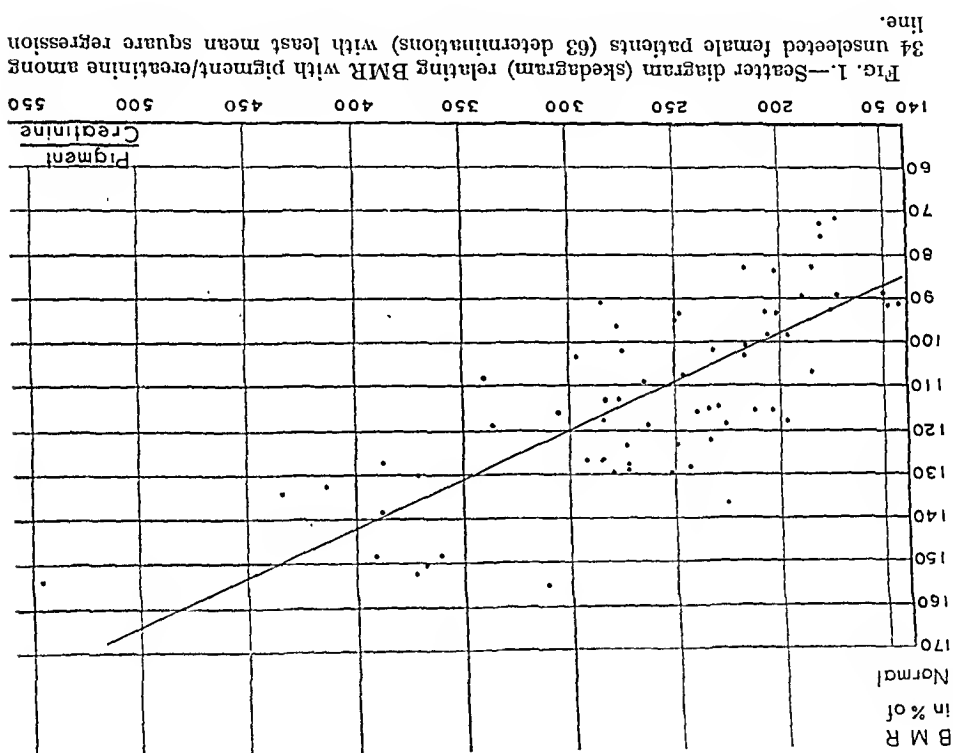


Fig. 1.—Scatter diagram (skedogram) relating BMR with pigment/creatinine among 34 unselected female patients (63 determinations) with least mean square regression line.

These studies constitute an extension and essential confirmation of Drabkin's earlier work.

**Summary.** 1. The hourly excretion of urinary pigment was constant in any given subject within a coefficient of variation of about 10%, whether measured for an overnight period or for a basal resting period. 2. The same constancy was true of the ratio of the concentration of urinary pigment to that of urinary creatinine. Moreover, among normal males, at least, there was a correlation between the rate of pigment output and the rate of creatinine output; in other words, the concentration ratio P/C was constant among normal males. 3. When the metabolic rate was elevated by the ingestion of thyroid extract, P/C variations paralleled the trend and the fluctuations of the BMR quite closely. 4. Among adult females there was a high correlation between individual P/C values of urine collected during a basal period and the

BMR determined at the end of that period. Among a much smaller number of adult males, the correlation was lower, but this group included a number of observations where the clinical impression was more consistent with the P/C value than with the widely divergent BMR. This circumstance requires further investigation.

It is believed that the P/C ratio applied to a large number of cases may show results of interest. It is also possible that this ratio may be useful as a substitute for the BMR determination when the latter cannot be performed or gives results which are inconsistent with the clinical diagnosis.

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# ELECTROENCEPHALOGRAPHIC STUDIES DURING FEVER INDUCED BY TYPHOID VACCINE AND MALARIA IN PATIENTS WITH NEUROSYPHILIS

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WITH the growth of experience in electroencephalography (EEG), it becomes increasingly evident that an alteration of the EEG may occur in a wide variety of conditions. As a result of our interest in the changes in neurosyphilitic patients occurring during antiluetic therapy, our attention was directed toward a study of the brain-wave patterns during fever. In the preliminary experiments, it soon became clear that striking modifications of the EEG were associated with the height of the fever paroxysm. The present paper is an analysis of the EEG changes during the rise and fall of temperature induced by malaria and/or intravenous typhoid vaccine.

**Material and Methods.** Twenty-three patients undergoing treatment in the Neurosyphilis Clinic of the Boston Psychopathic Hospital were utilized in this study. The diagnostic classification and number of cases were as follows: general paresis, 13 cases; meningo-vascular syphilis, 3; meningo-vascular syphilis with probable general paresis, 2; tabes dorsalis, 2; optic atrophy, 2, and tabo-paresis, 1. The individual cases varied considerably in the duration and severity of the clinical picture.

Fever was induced by intravenous administration of graduated doses of typhoid vaccine or by inoculation with malaria. The individual doses of typhoid vaccine varied between 50 and 250 million killed suspended typhoid organisms. EEG tracings were obtained before the temperature rise and at intervals of  $\frac{1}{2}$  to 1 hour during the fever. Temperature was taken before and after each



brain-wave recording. Fluids were given freely as requested by the patient and care was taken to keep the patient awake and as comfortable as possible during the period of observation.

Observations on 54 paroxysms of fever were made, 34 of which were induced by typhoid vaccine in injection and 20 by malaria.

Malarial paroxysms were generally ushered in by a more severe chill than were those resulting from typhoid vaccine injection. In addition the rise in temperature with malaria was usually more rapid and the temperature peak higher. As a rule, the more sudden and more severe the fever paroxysm by either method of induction, the greater was the subsequent modification of the EEG. For simplicity the data for both methods will be described together.

**Apparatus.** A Grass 6-channel ink-writing amplifier with a paper speed of 30 mm. per second was employed. Electrodes were applied to frontal, motor, and occipital areas of the right hemisphere, and indifferent electrodes were applied to the mastoids. Simultaneous monopolar and bipolar tracings were recorded. All records were obtained at a standard amplification, and with the use of a filter allowing undistorted reproduction of wave forms up to 50 cycles per second.

**Observations.** The basic pattern of the patients before fever varied considerably in type. Eight were considered to be within normal limits, 5 were classed as borderline, and 10 as abnormal. The findings were in general agreement with those of Finley, Rose and Solomon<sup>4</sup> who, in a larger group of patients with neurosyphilis, found that the majority of tracings belonged to the borderline-abnormal group.

EEGs taken at intervals between the time of injection of the typhoid vaccine and the development of fever showed no essential change.

During the chill, in the case of malaria as well as typhoid, the EEG showed evidence of marked muscular activity. Shivering, and particularly chattering of the teeth, was associated with rapid spikes varying in frequency between 15 and 20 per second (Fig. 1). To some extent both the shivering and the spike potentials could be controlled voluntarily by the patient.

There was a grossly detectable alteration in the EEG during the rise in temperature in 22 of the 23 patients, and the alteration was almost always progressive. The nature of the alteration in the majority of records could be summarized as follows (Figs. 1 and 2): (1) An increase in the irregularity of the pattern. (2) An increase in the number of slow or delta waves. (3) An increase in the voltage of the potentials. A few of the fever tracings contained both rapid and slow activity, but with the latter dominating the record.

The rise in temperature was associated with progressive loss of the characteristics of the patient's basic electrogram, and the pattern at high temperatures ( $104^{\circ}$  to  $105^{\circ}$  F.) was utterly different from that at the control temperature. With the decline of fever there was a gradual resumption of the original characteristics. When the patient was entirely afebrile, the electrical pattern was again similar to that of the prefebrile period.

There were variations in the magnitude of changes during fever which were to a large extent in accordance with the severity of the paroxysm—that is, the rapidity of the rise of fever and its final height. The more rapid the rise and the greater the final elevation of temperature, the more marked the changes in the EEG.

In addition, the more severe the clinical picture of the disease, the more marked were the changes during the fever. During fever, the EEG showed considerable individual variation in the amount of delta activity. In some cases the delta activity appeared at random throughout the record, in other cases the delta activity was

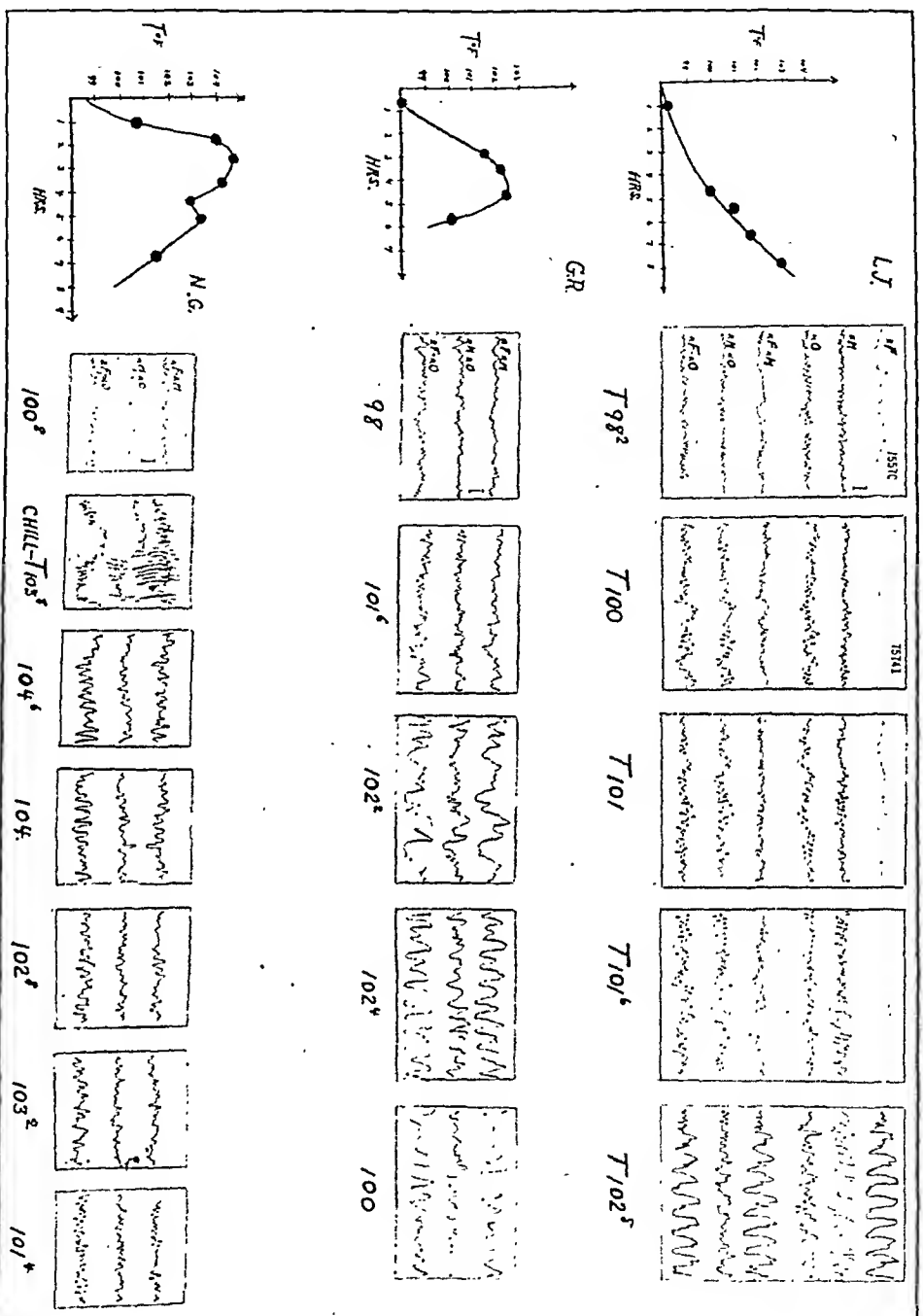


FIG. 1.—EEG changes during fever. Tracings during fever in 3 patients with neurosyphilis. The dots on the graph at the left represent points on the fever curve at which the tracings to the right were taken. All leads are from the right hemisphere. The records of L.J. and G.R. are of 3 seconds duration and are both monopolar and bipolar. The records of N.G. are of 2 seconds duration and are bipolar only. The upright calibration line in the first tracing of each series represents 50 m.v. Note that changes in the EEG parallel the rise and fall of temperature. Patient L.J. showed progressive slowing of the waves and increase of voltage with the rise of temperature until at 102.8° F, bursts of high voltage 2 to 3 per second slow waves appeared, many with spike and wave configuration. Patient G.R. showed 2 to 3 per second high voltage delta activity at 102.4° F, with considerable slow activity remaining even at 100° F. Patient N.G. shows rapid spikes due to the chill at 103.5° F with relatively little slowing of the cycles even at 104° F.

continuous, and in still other cases the delta activity was "organized" into bursts of high voltage slow cycles, 2 to 3 per second (Fig. 1). The episodic slow rhythm during fever could not have been anticipated from the control tracing, although the latter might have been abnormal (Fig. 1). Some of the records during fever suggested strongly the type of activity seen in epilepsy and allied disorders.

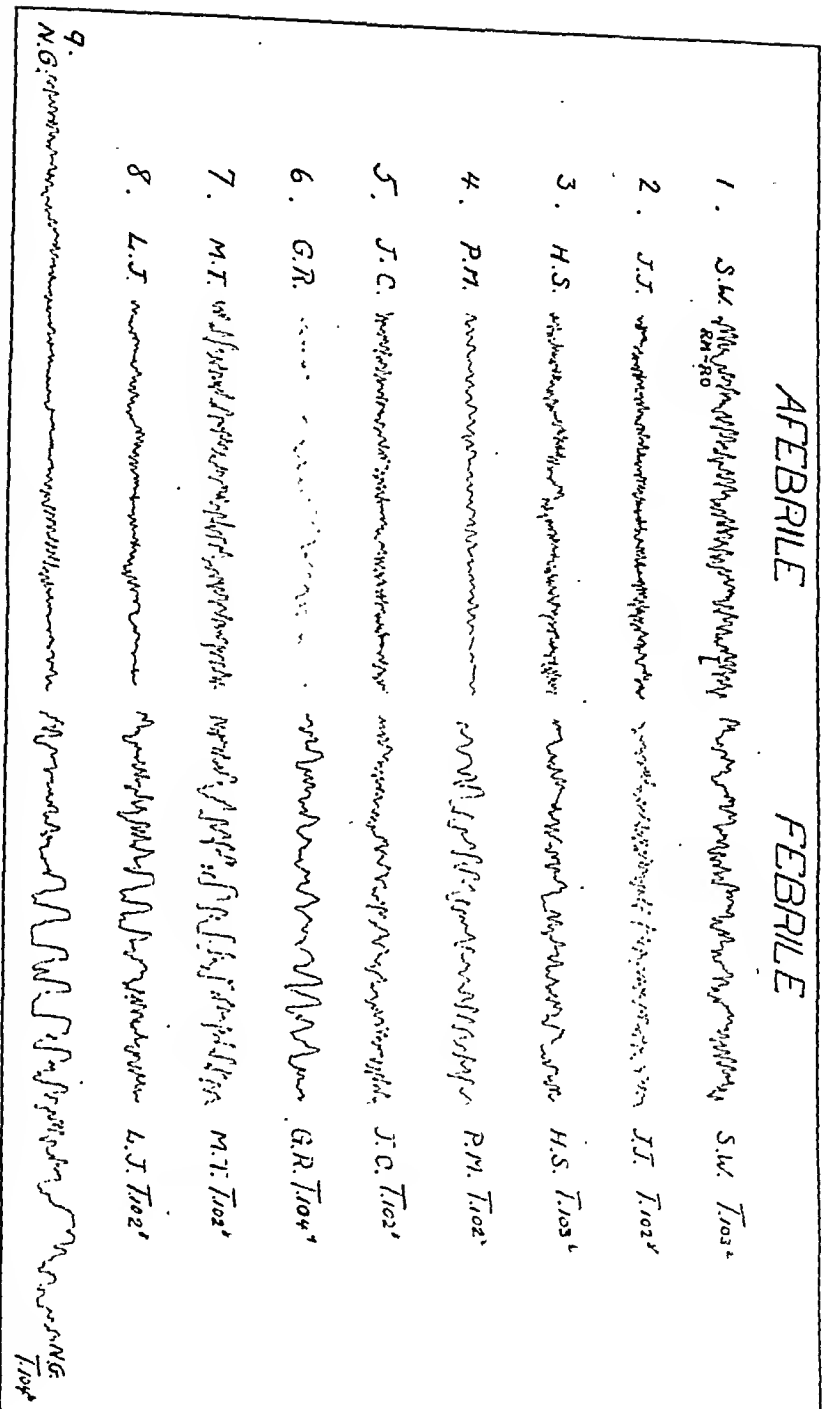


Fig. 2.—Nine varieties of brain-wave patterns in patients with neurosyphilis and the corresponding patterns during fever. The patterns of Nos. 4, 5, 6, 7, 8, and 9, during fever showed "episodes" of slow waves in groups. The tracings are all bipolar from the right motor to right occipital area. The length of the upright line in the upper left tracing is equal to 50 microvolts. All tracings are of 5 seconds duration except No. 9, which is of 7 seconds duration.

There was considerable individual variation in the rate of return of the EEG to normal during the decline of fever. Comparison of records at a given temperature during the decline of fever and during the rise of fever revealed that the two were not identical. In some patients there was a relatively abrupt return to the basic characteristics of the EEG in the early defervescence of fever, and this was associated with

TEMP TIME  
98°F 10:30 A.M. RM-RO  
QUIET COOP I

104° 12:00 Noon EXHAUSTED  
QUIET COOP AFTER CHILL

105° 1:00 P.M. QUIET COOP  
CONFUSED

105° 1:30 P.M. QUIET COOP  
PERSPIRING

105° 2:30 P.M. QUIET COOP  
LETHARGIC

104° 3:15 P.M. PERSPIRING  
PROFUSELY  
FEELS AND LOOKS  
BETTER

102° 4:50 P.M. PERSPIRING  
MENTALLY MORE  
ALERT

Fig. 3.—EEG during malarial paroxysm. Tracings taken at intervals on a patient with neurosyphilis during a malarial paroxysm. Each strip equals 3 seconds. All are bipolar records from right motor to right occiput. Vertical line equals 50 microvolts. At 98.4° F., the record is all smooth continuous alpha rhythm of normal frequency. At high temperatures the record is of higher voltage, contains considerable slow activity and is very irregular. At 105.4° F., large slow swings appear which partially obliterate the brain potentials. These swings are associated with marked perspiration. Note that at 104.4° F., as the fever breaks, the pattern is different from that at 104.6° F., on the rise of temperature, and that 102.8° F., it is already very similar to the control at 98.4° F. The relatively abrupt improvement of the record following the break of fever was associated with subjective improvement of the patient.

clinical evidence of recovery from the febrile reaction. At this more or less critical period in the febrile course there was, in addition to abrupt "improvement" of the EEG, marked perspiration, together with increased alertness and responsiveness and a subjective feeling of well-being (Figs. 3 and 4). During fever several patients showed significant clinical changes in the form of restlessness, irritability, listlessness, and confusion. Apathy

and listlessness followed regularly after marked exhausting rigor. Confusion appeared particularly when the temperature was very high in patients whose orientation previously was not clear. These patients, by and large, showed the most changes in their EEG tracings.

**Discussion.** Knowledge regarding the changes of the brain potentials in various conditions is of importance in understanding the physiology of the brain and in improving the interpretation of the EEG in clinical conditions. Fever is a dramatic phenomenon inviting interest and frequently yielding information of fundamental significance.

TEMP TIME  
99°F 1:30 P.M. RN-Ro  
QUIET COOP  
GOOD SPIRITS  
CLINICAL COND

101° 2:00 P.M.  
QUIET COOP  
GOOD SPIRITS

102° 2:30 P.M.  
QUIET COOP  
HEADACHE

103 3:00 P.M.  
CLOUDED  
CONFUSED

102° 3:30 P.M.  
PERSPIRING  
MENTALLY CLEARER  
MORE ALERT

102 4:10 P.M.  
PERSPIRING  
RESTLESS

Fig. 4.—EEG during fever produced by typhoid vaccine. Tracing taken at intervals on a patient with neurosyphilis during a paroxysm following intravenous typhoid vaccine injection. Each strip equals 5 seconds. All are bipolar records from right motor to right occiput. Vertical line equals 50 microvolts. During fever, high voltage slow activity dominates. At 102.2° F. on the downgrade of fever, the pattern is much more like the control (99.6° F.) than at 102.2° F. on the upgrade of fever. Relatively abrupt improvement in the brain waves during early defervescence was associated in this case with clinical improvement.

The main feature of the records in this study is an increase in the slow or delta activity during fever. The higher voltage found is that usually to be expected in association with slow activity, and the irregularities are mainly an expression of mixed frequencies. In the fever records, the irregularities are to a large extent due to the addition of slow waves to the basic pattern.

What is the cause of the delta activity? Is it the fever? To be sure, the amount of delta activity is in general correlated with the height of the temperature, but the fact that at a given temperature in the rise of fever the record does not necessarily show identical delta activity as at the same temperature in the fall of fever, indicates that the temperature *per se* is not the whole explanation.

That the increased metabolism in fever is not the sole reason for the alteration in brain potentials is suggested by the reports of increased frequency of brain waves in patients who as a result of thyrotoxin or dinitrophenol administration develop a rise in basal metabolic rate.<sup>8</sup> Also, it is reported that patients with hyperthyroidism generally have increased wave frequency, while patients with myxedema generally show decreased wave frequency.<sup>7</sup> Although the records obtained during fever show predominantly decreased wave frequency or slow wave activity, occasionally there is admixture of cycles more rapid than normal. It is possible that several influences, of which the metabolic rate is one, play a rôle in the alteration of the brain-wave pattern during fever.

Does the presence of central nervous system disease play an important rôle in the appearance of delta activity? The rough correlation between the severity of the disease process and the delta activity during fever at first suggested the possibility that, in non-syphilitic patients, the delta effect might not be obtained. However, from a few unpublished experiments it appears that the slow wave activity is by no means limited to patients with neurosyphilis. Thus far, we have observed it during fever following intravenous typhoid injection in 2 patients with schizophrenia and 1 patient who had recovered from acute alcoholic hallucinosis. Another patient with pyrexia of unknown etiology (and with negative tests for syphilis) demonstrated also increased slow waves during fever.

Does the type of induction of fever play a rôle in the appearance of delta activity? Hoagland<sup>5</sup> reported that in fever induced by diathermy in 10 patients (6 with neurosyphilis) there was an increase in frequency of the brain waves. Berger,<sup>2,3</sup> on the other hand, observed slow waves during fever in 2 tabetic cases, one treated with malaria and one with "pyrexifer." In our cases, both typhoid and malaria acted similarly; that is, increased delta activity was associated with fever. These data suggest that the method of induction is possibly to be taken as a significant factor. We should point out, however, that until a great deal more is known about the phenomenon of fever and its effect on the brain potentials, it is unwise to speak of the effects observed as caused by the fever. Alteration of temperature from any cause is only one manifestation of many disturbances of body physiology.

One striking feature of the records is that the delta activity may be sporadic, continuous, or organized into bursts of 2 to 3 per second slow cycles. Several runs of very suggestive spike and wave activity (L.J., Fig. 2) were observed in a patient without a history of seizures. Records with sporadic or continuous delta are seen frequently in cases with intracranial pathology, and records with episodic slow activity are seen frequently in epilepsy and allied disorders. This raises interesting problems: (1) the possibility that fever is a method of eliciting abnormal activity which does not occur in the basic pattern; and (2) that the abnormal activity is related to the epileptic predisposition of the patient or to intracranial disease. Patients with neurosyphilis frequently develop convulsions during the course of

their illness, and fever may precipitate convulsions in non-epileptic children and some adults. On the other hand, if correlations with epilepsy and intracranial pathology are not established, this finding will serve to emphasize the lack of specificity of special wave forms as indicators of specific diseases.

**Summary.** Twenty-three patients with neurosyphilis were subjected to 54 bouts of fever induced by intravenous typhoid vaccine injection and therapeutic malaria, and EEGs were taken before and at intervals during the fever. As the temperature rose there was a gradual increase in the amount of delta activity in the voltage and in the irregularity of the brain potentials, and as the temperature subsided there was a gradual return to the subject's prefebrile pattern. The magnitude of the changes were correlated with the severity of the fever paroxysm and with the severity of the disease. The delta activity appeared as sporadic slow waves, continuous slow waves, and as organized bursts of slow activity. The factors of temperature, rate of metabolism, type of disease, and type of induction of fever are considered as possible causal factors in the production of slow activity.

We are indebted to Miss Marie Healey and Mrs. Janet Lavery for able technical assistance.

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### THE USE OF EQUINE GONADOTROPIN IN MALE INFERTILITY\*

#### AN ANALYSIS OF 127 PATIENTS

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The treatment of male infertility, which began with the earliest realization of the husband's responsibility in a barren marriage, was given its greatest impetus with the discovery of the pituitary gonadal relationship. This discovery led to the introduction of human pregnancy urine and of menopausal urine as potential spermatokinetic agents. The results obtained with the use of these products were not satisfactory. The extract of pregnant mare serum (equine gonadotropin) was then introduced and it, in turn, has received wide therapeutic trial. Although this substance was found to possess unusual

\* The author is indebted to Dr. David R. Meranze, Director of Laboratories of the Mount Sinai Hospital, for his aid in interpreting the histologic sections presented herein.

spermatokinetic qualities in the experimental animal, the paucity of clinical reports reflects the lack of good results in the human. Our own clinical experience with equine gonadotropin has been equally disappointing. Although the results are somewhat better than those obtained with the products previously employed, the stimulative properties of even these gonadotropins do not measure up to experimental expectations. It is our purpose in this report to analyze the results of treatment with equine gonadotropin, to examine critically the lesions associated with the semen deficiencies and to deduce therefrom the reasons for failure of treatment. Is the gonadotropin which was potent experimentally, ineffective in the human; or are we dealing with lesions of the human seminiferous tubules which are different from those found or induced in the experimental animal? Does the administered gonadotropin have insufficient spermatokinetic qualities clinically, or is the lesion of the testis of such a character that the germinal epithelium is no longer capable of responding to stimulation?

This report is based on material gathered over a period of nearly 4 years. The conclusions as to the ineffectiveness of equine gonadotropin are based on an analysis of 230 consecutive patients treated with this substance. However, 103 of these patients either received other treatment in addition to the gonadotropin, or were insufficiently observed or inadequately treated. The final analysis is therefore based on a review of the results in 127 patients with faulty spermatogenesis treated with equine gonadotropin only. The preliminary studies done on the 127 patients included not only a physical examination and the usual laboratory tests, but a testicular biopsy as well. Urine studies for the determination of androgen and gonadotropin content were done only in the few instances in which they were especially indicated. The determinations were inconclusive and are not included in this report. The equine gonadotropin\* was administered intravenously. The dose employed in 119 patients was 400 I.U.† administered 3 times weekly for a period of from 3 to 6 months. Seven patients were given 800 I.U. and 1 patient 2000 I.U. 3 times weekly. The results of treatment with equine gonadotropin in the 127 patients are summarized in Table 1. Of the 127 men treated, 12 were "cured," 22 were "improved," and 93 remained unchanged. The terms "cured" and "improved" refer to semen changes only. As a criterion of cure, a total count of at least 200 million spermatozoa was arbitrarily chosen.† Any increase of the sperm count, such as increases from an initial 2 million to a post-therapeutic 3 million, were considered as variations having no relation

\* Anteron, generously supplied by the Schering Corporation.

† I.U., International unit. An international unit is "the specific gonadotropic activity of 0.25 mg. (250 gammas) of the standard preparation in possession of the Health Organization of the League of Nations." One rat unit (Carliland-Nelson) equals 16 I.U. ‡ This figure is to be compared with a total of 350 million spermatozoa, generally accepted as average normal.



to treatment and were therefore classified as unchanged. Ultimate pregnancy in the wife was not accepted as a criterion. Patients with large increases in the sperm count were classified as cures even though pregnancy did not occur. Conversely, one with a constantly low sperm count was classified as unchanged even though his wife became pregnant during the course of treatment.

No attempt is made to divide the semen deficiencies into the usual clinical groups, *viz.*, azoospermia and oligozoospermia, since it is our contention that the two have similar etiologies and that the difference between them is merely one of degree. Similarly, in this short résumé, the individual sperm counts are not listed in detail. It is to be noted, however, that better results were obtained in patients who had the higher sperm counts at the beginning of treatment. This observation is quite significant and will be dealt with below.

**The Scope of Testicular Biopsy.** Up to the present time, failures of treatment have been generally attributed to inadequacy of the administered gonadotropin. The nature of the lesion involving the seminiferous tubules (and accounting for the reduced spermatozoan population) has received little attention. No one has up to now seriously considered the possibility that a very large proportion of these lesions do not result from gonadotropin deficiency and can therefore not be repaired by gonadotropin administration. Generally the pretherapeutic classification has consisted of a differentiation between oligozoospermia and azoospermia. Patients with non-obstructive azoospermia have generally been given a poor prognosis, whereas those with oligozoospermia have been encouraged to take treatment on the supposition that the few spermatozoa in the ejaculate indicated healthy but incompletely matured seminiferous tubules, each of which had failed to yield its maximum quota of spermatozoa.

Fortunately, the lesions affecting the seminiferous tubules are subject to close scrutiny. This is accomplished by means of testicular biopsy. In 1940,<sup>1</sup> we reported the use of testicular biopsy in the diagnosis of male infertility. More recently,<sup>2</sup> we described a variety of testicular lesions found on biopsy in instances of faulty spermatogenesis. A satisfactory classification of these lesions is still not available. Many more histologic sections require careful study before an acceptable and workable classification is made. For the present, the following clinico-pathologic groupings, based on the severity and the relative reversibility of the lesions, are presented because of their usefulness in prognosis. Of the 4 groups listed below, only Group 3 contains underfunctioning seminiferous tubules which may be expected to regenerate under favorable conditions.

1. *Complete Atrophy.* The seminiferous tubules are lined by a single layer of undifferentiated cells. The basement membrane is often wrinkled from shrinkage and may or may not be thickened. The semen contains no spermatozoa (Fig. 2).
2. *Severe Degenerative Changes.* Atrophy is incomplete. The seminiferous tubules are lined with 2 or 3 layers of germinal epithelium. Cellular vacuolization is widespread. Maturation is nowhere present. The basement membrane of the tubule may be normal in appearance but very often is thickened.

and fibrotic. This fibrosis may be so extensive that the ring it forms is thicker than the tubule itself. In many instances, round cell infiltration is present as evidence of inflammation. The semen contains no spermatozoa (Fig. 3).



Fig. 1.—Normal, showing highly cellular, regularly organized elements. (X 250.)

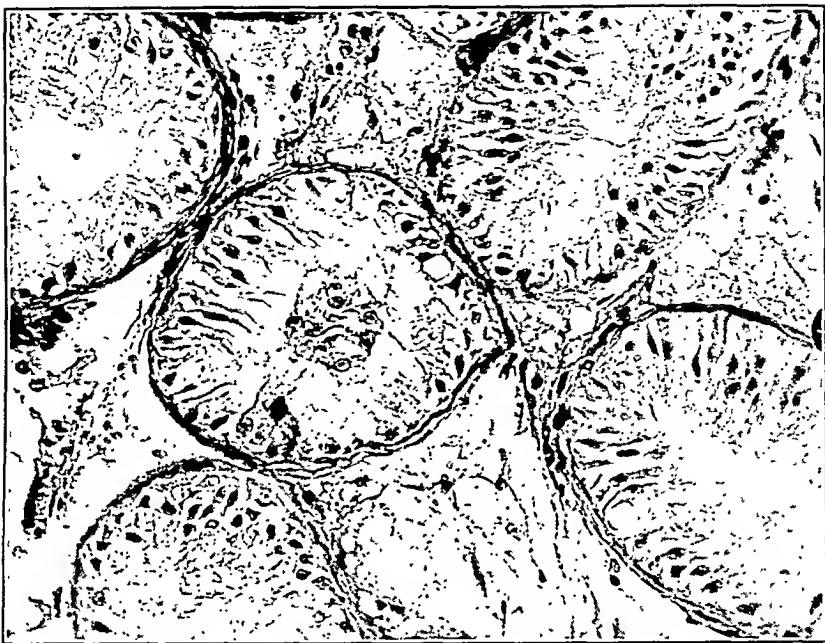


Fig. 2.—Complete atrophy. Only a single layer of undifferentiated cells is present in most tubules. There is slight peritubular thickening. (X 250.)

3. *Moderate Degenerative Changes.* The pathologic process is less advanced than in group 2. The seminiferous tubules contain more cellular layers. Evidence of maturation is now present though not widespread. Cellular



Fig. 3.—Advanced degeneration. The tubules contain 2 or 3 layers of seminiferous epithelium. No evidence of maturation. (X 250.)

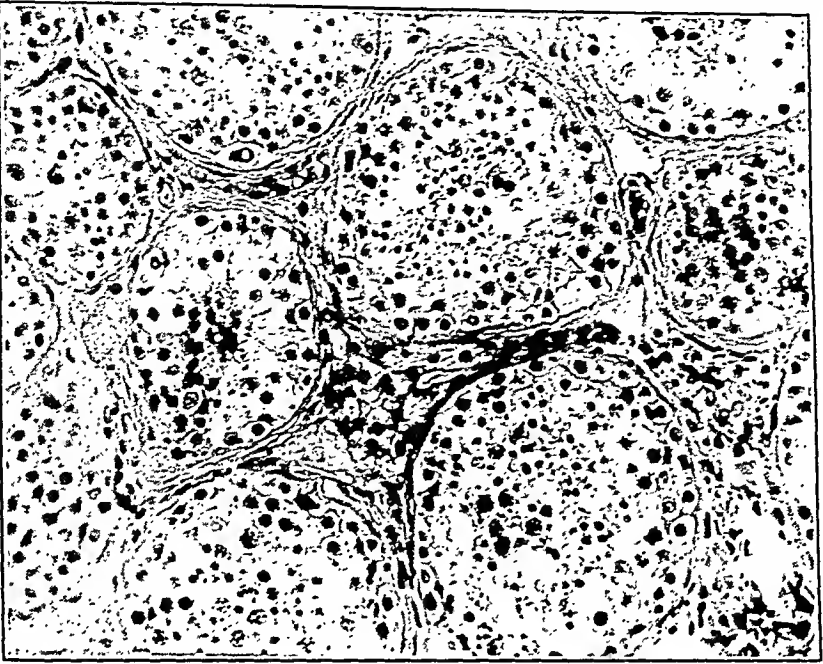


Fig. 4.—Moderate degeneration. Note disorganization of cellular elements even in the highly cellular tubules. Compare with Figure 1. Two of the tubules reveal some maturation. Sperm count 8,200,000 per cc. (X 250.)

vacuolization is common. A great many of the tubules have only 1 or 2 layers of cells, their lumina being filled with cellular debris. The basement membrane is usually normal, but peritubular fibrosis may be present. The semen con-



Fig. 5.—Islands of relatively normal tubules surrounded by atrophic ones. (X 250.)

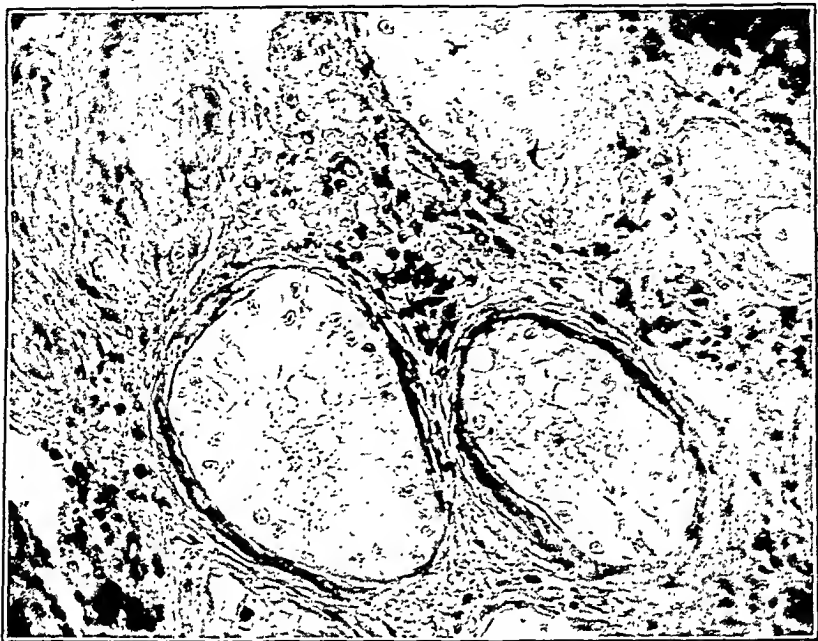


Fig. 6.—Failure of development—characterized by atrophic tubules and an increase in interstitial tissue—chiefly of connective tissue elements (cells with dark staining elongated nuclei). Note relative sparsity of cells of Leydig. (X 250.)

tains some spermatozoa, the number depending on the prevalence of matured

4. *Combinations of Groups 1, 2 and 3.* The healthier tissue is usually arranged in small islands which are surrounded by tubules displaying the various degenerative phases already described. The islands may contain healthy, matured tubules or even these may show evidence of underdevelopment or degeneration. The surrounding tissue reveals the more advanced tissue changes. Spermatozoa are found in the ejaculate only if there are matured tubules in the islands (Fig. 5).

As already stated, this classification is presented merely to list the lesions according to their severity, thus attaching to them a prognostic significance. In addition, there are observed, at all levels of severity, certain pathologic features which appear to have an etiologic significance. These are:

1. In instances of atrophy or severe degeneration the size of the tubules may be normal or reduced. It is assumed that in these cases the size of the tubules is an index of the stage at which development ceased and degeneration set in. Thus, small atrophic tubules indicate failure of development, whereas atrophic tubules of normal size point to degenerative changes which set in after development was more or less advanced.

2. At any of the levels of degeneration, the presence of peritubular fibrosis is indicative of a long-standing pathologic process. If accompanied by round cell infiltration it is definite evidence of a previous inflammation, even in the absence of a clinical history of orchitis. 3. The character and quantity of the interstitial tissue is significant. There is apparently an increase in the amount of normal interstitial tissue (but not necessarily in the interstitial cells of Leydig) in instances of clear-cut endocrine underdevelopment. This is a more or less constant feature and is observed even when the seminiferous tubules are normal in size. It may serve to differentiate between an endocrine and non-endocrine etiology (Fig. 6).

This classification would be of little practical importance if the correlation between the biopsy and semen findings were constant. But that is not the case. Tubules which yield no spermatozoa may be completely atrophied (Group 1) and irreparable, or they may reveal only moderate degenerative changes (Group 3) which are reversible. An individual with a very low sperm count may possess underdeveloped tubules most of which can be stimulated to growth under favorable conditions, or he may have small islands of mature tubules interspersed among tubules showing extensive atrophy (Group 4). These mature tubules are already functioning at full capacity and attempts at further stimulation are useless. Another patient with a moderately reduced sperm count may possess a large number of irreparably degenerated seminiferous tubules surrounding normally functioning ones (Group 4), or he may reveal healthy tubules which are somewhat immature and require only adequate stimulation by gonadotropins (Group 3). Furthermore, testes whose function has been impaired by a previous inflammation, as evidenced by peritubular fibrosis and round

cell infiltration, are not likely to respond to any type of stimulation, not even to the administration of gonadotropins of adequate potency. Finally, one may find an oligozoospermia in an individual whose biopsy reveals an abundance of normally functioning tubules. Under such circumstances, the reduction in the sperm count is associated with evidence of seminal vesicular infection and impaired drainage through the ejaculatory ducts. In such cases, treatment should be directed toward elimination of the infection rather than to administration of gonadotropins. With the introduction of sulfonamide therapy, the percentage of "cures" in this group has been greatly increased. The results of this form of therapy are soon to be reported in a separate communication.

TABLE 1.—RESULTS OF TREATMENT WITH EQUINE GONADOTROPIN

No. of patients	"Cured"	"Improved"	Unchanged
127	12 (9.3%)	22 (17%)	93 (73.7%)

Thus, these studies serve to emphasize the importance of testicular biopsy not only in evaluating the degree of damage to the seminiferous tubules but also in determining the correct indication for gonadotropin therapy. Moreover, a second biopsy following a given period of treatment with gonadotropins yields definite information relative to the efficacy of the administered product, especially in those instances in which reexamination of the semen fails to show any improvement.

**Evaluation of Gonadotropin Therapy.** The results of treatment with equine gonadotropin are obviously poor. One must keep in mind, however, that this is an analysis of consecutive unselected patients and that it is not therefore a fair estimate of the value of equine gonadotropin when it is administered only to patients in whom it is indicated. An explanation for the lack of response to the gonadotropins is found in the data uncovered by testicular biopsy in the 93 patients in whom repeated semen examination revealed no improvement. In 48 of these patients the biopsies preliminary to treatment revealed any one of the irreparable lesions illustrated above, the most prominent of which was peritubular fibrosis. This was very often associated with round cell infiltration, indicating an inflammatory origin. These lesions were obviously not produced by an endocrine deficiency and could therefore not be repaired by endocrine administration. After a trial period of treatment with gonadotropins, biopsies were repeated in 4 of these patients. Examination of the histologic sections failed to reveal any growth of the germinal epithelium.

The remaining 45 patients revealed lesions which were potentially reversible. These lesions could conceivably have been produced by gonadotropin insufficiency, although even some of them may have had other etiologic backgrounds. Of these 45 patients, testicular biopsies were repeated after a test period of treatment in 15. Of these 15, 11

revealed increased growth of the germinative epithelium of the tubule, even though the semen picture remained unchanged (Table 2).

TABLE 2.—ANALYSIS OF FAILURES. CORRELATIONS OF RESULTS OF TREATMENT WITH BIOPSY FINDINGS

Histologically irreparable lesions	Histologically irreparable lesions	Number of patients with semen improvement	Number of patients with improved histologic picture after treatment
45 (48%)	48 (52%)	0	0
0	4	15	11 (73%)

However, this epithelial growth seemed to be limited to a multiplication of the younger cell forms. Cell division occurred, but maturation did not follow. The failure to produce actual maturation of the germinative epithelium may have resulted either from the fact that the administered gonadotropin lacked a maturation fraction or that it was not sufficiently potent and should have been administered in larger dosage. The answer remains just as obscure when the data of the 12 "cured" patients are examined. With 2 exceptions the lowest total sperm count before treatment was 50 million. Testicular biopsies in these patients uniformly revealed a good number of actively functioning seminiferous tubules. Even those tubules which were not normal contained several layers of differentiated epithelial cells which would conceivably require little stimulation for maturation. The widespread atrophy, noted in some of the advanced oligozoospermias, indicating a poor prognosis, was absent.

**Summary and Conclusions.** 1. Results in a series of 127 *unselected* cases of male infertility treated with equine gonadotropin are unsatisfactory, although the substance appears to have therapeutic value in selected cases.

2. The various histologic pictures of the seminiferous tubules found on testicular biopsy in infertile men are herein described. Detailed study of the histologic sections of the patients who improved and of those who failed to respond to treatment shows a remarkable consistency in the ability to prognosticate the outcome from testicular biopsy.

3. The percentage of patients who reveal irreparable lesions of the seminiferous tubules is surprisingly high (52%). It is useless to employ gonadotropin therapy in these individuals.

4. Of the patients with faulty spermatogenesis 48% revealed seminiferous tubules which could conceivably regenerate. The failure to obtain an equally large percentage of "cures" is either the result of insufficient potency of the administered gonadotropin or of the lack of a maturation fraction therein.

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# ANALYSIS OF RESULTS OBTAINED WITH SMALL DOSES OF GOLD SALTS IN THE TREATMENT OF RHEUMATOID ARTHRITIS\*

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In 1929, Forestier<sup>1</sup> reported favorable results with injections of gold salts in the treatment of arthritis. Many reports have appeared since then and the consensus of opinion is that the method has a definite place in the treatment of rheumatoid arthritis even though there may be toxic reactions which may even be fatal. The mechanism underlying the beneficial effect and the toxic reactions is unknown, the dosage and method of treatment still being given on an empirical basis. The number and severity of toxic reactions which occur with the commonly recommended dosage and the unscientific basis for this dosage made it desirable to investigate the subject further.

This study was begun in 1940 and was given further impetus by Hartung's<sup>2</sup> findings that the bacteriostatic property of the sera of patients who had received 150 mg. of gold salts was similar to that of patients who had received 900 mg. in the same period of time (6 weeks). Later, Freyberg<sup>3</sup> published the results of his study on the concentration of gold in the plasma and its elimination in urine, feces, etc. He concluded that: (1) With increasing doses of gold, there was a general tendency toward an increase in the blood gold level and in its urinary excretion, followed by a leveling-off, even though greater retention may have been accruing. (2) When injected weekly, the amount of gold eliminated did not exceed 25% of that injected at any one time. (3) Plasma values were highest 1 hour after the injection, but remained above normal for 24 hours, varying with the amount of gold injected. (4) The higher the dose, the greater the urinary excretion of gold. (5) Gold was found in the blood and urine as long as 9 to 12 months after injections, the length of time being approximately proportional to the size of the weekly dose. After a weekly dose of 12.5 mg., gold was found in the blood and urine for 1 month; after 25 mg., it was found for 3 months; and after 50 mg. for 6 to 10 months. (6) Toxic reactions were much more frequent in

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patients who received large weekly injections, the severity usually being proportionate to the amount of gold injected. (7) With smaller doses, the same therapeutic results were obtained as with the conventional larger doses.

The studies of these 2 investigators further strengthened our belief that the conventional dosage of gold salts was unsuitable.

**Materials and Methods.** Gold thioglucose,\* which contains 50% of gold, was given intramuscularly to 100 patients with rheumatoid arthritis who had high sedimentation rates. The interval and dose varied from time to time to determine the minimal effective dosage.

**Dosage.**† From preliminary experiments it appeared that small doses would give satisfactory results in rheumatoid arthritis. The tentative schedule adopted was 5 mg. intramuscularly twice a week for 3 weeks; 10 mg. twice a week for 3 weeks; and then 25 mg. once a week. If toxic symptoms did not develop after 3 weeks of the latter dose, they rarely appeared subsequently. Most of the patients were given 25 mg. a week throughout the series, but in a few cases that showed no improvement after 4 weeks, the dose was increased by 5 mg. every 2 weeks until improvement occurred or until 50 mg. a week was reached. For example, if improvement occurred on 35 mg. a week, the dose was not increased further. However, if the improvement did not continue the dose was again increased but never above 50 mg. We did not give a definite course of injections but continued gold salts indefinitely, or for at least 12 months. In this way we were able to prevent the relapses that so frequently occur with the standardized course method.

In some instances, the initial dose was even less than 5 mg. a week. In patients with severe rheumatoid arthritis with very high sedimentation rates, moderate to severe anemia, marked loss of weight, insomnia, and all the signs of active infection, or in older people, the dose was only 2 or 3 mg. twice a week for the first 4 weeks, then 5 mg. twice a week for 4 weeks, 10 mg. twice a week for 4 weeks, and finally 25 mg. a week, as tolerated. However, a fixed dosage schedule is undesirable and every patient must be studied individually.

**Toxicity.** Toxic symptoms following this therapy fell into the following groups: cutaneous (localized dermatitis, dermatitis exfoliativa, urticaria, etc.), mucus membrane involvement (ulcers in mouth and throat, gastritis, enteritis, vaginitis, etc.), renal and hepatic. With the small doses employed in this study, 42% developed toxic symptoms, 50% of which occurred before the patients had received 100 mg. of this gold preparation and 86% before they had received 200 mg. If toxic symptoms did not appear by that time they seldom occurred subsequently, and larger doses, up to 50 mg. per week, were usually tolerated without unfavorable effect. Patients receiving the smaller doses quickly recovered from toxic symptoms, the severity and duration being proportionate to the size of the weekly dose and the total amount of gold injected. This confirms the findings of Freyberg.<sup>2-3</sup>

As small a total dose as 20 to 30 mg. occasionally was followed by toxic symptoms. However, they usually were mild and disappeared after a few weeks. Four patients developed toxic symptoms of doubtful classification. One developed edema of both legs which disappeared

\* Solganal-B Oleosum, the material used in this study, was furnished gratis by Schering Corporation.  
† All dosages given in this paper are in terms of gold thioglucose rather than in terms of gold.

after the treatment was discontinued, but returned when the injections were resumed. The urinalyses were negative, and we were unable to account for the effect. Another who was quite ill developed severe hicoughs, apparently secondary to hepatitis, which persisted for 3 weeks. A third patient had a severe, prolonged headache not

TABLE 1.—THE RELATIONSHIP OF TOXIC REACTIONS TO THE DOSE OF GOLD THIOGLUCOSE

Type of reaction	Total dose (mg.)	Average dose weekly (mg.)	Interval (weeks)	Duration of toxic symptoms (weeks)					
					Cutaneous (17):	Dermatitis exfoliativa	Localized dermatitis	Maculo-papular eruptions	Urticaria
	655	25	26	13					
	730	24.33	30	10					
	200	20	10	7					
	70	16	3	4					
	130	20	8	5					
	80	11	7	4					
	60	8.5	7	5					
	110	9	13	6					
	100	10	10	3					
	80	8	10	2					
	90	10	9	2					
	40	7	6	2					
	70	8	9	3					
	25	6	4	2					
	80	10	8	3					
	20	10	2	2					
Angioneurotic edema	20	10	2	2					
					Mucous membrane involvement (11):				
Pharyngitis	20	5	4	2					
Vaginitis	145	14.5	10	4					
Stomatitis	130	16	8	5					
	130	16	8	5					
	40	7	6	1					
	20	5	4	1					
	80	11	7	2					
	90	10	9	4					
	120	10	13	5					
	200	20	10	6					
Gastritis	200	20	10	8					
Colitis	200	20	10	8					
					Liver involvement (2):				
Hepatitis	200	20	10	6					
Hepatitis with hicoughs	220	20	11	5					
					Kidneys (5):				
Hematuria	190	9.5	20	4					
Albuminuria	210	21	10	5					
	80	11	7	5					
	100	12	8	7					
	150	15	10	8					
					Hematologic (2):				
Reduction in platelet count	20	7	3	2					
	80	20	4	3					
					Central nervous system involvement (1):				
Encephalitis with vertigo	240	24	10	5					
					Type undetermined (4):				
Headache	135	11	12	3					
	160	20	8	5					
Vertigo	130	13	10	4					
Edema of legs	80	11	7	3					

relieved by the usual remedies, which was thought to have been the result of gold toxicity. Two who had vertigo were confined to bed for 1 and 2 weeks. They developed moderate anemia, complained of drowsiness, anorexia and nausea, and generally appeared to be quite ill. The symptoms could only be accounted for on the basis of gold encephalitis.

If large doses had been given in these 4 cases, the symptoms undoubtedly would have been more prolonged and more serious, and a fatality probably would have occurred in the patient with the more severe vertigo, drowsiness, etc. All other patients in the series, except those with dermatitis exfoliativa, recovered rapidly and did not appear to be seriously ill at any time.

When any toxic or doubtful symptoms appeared, the gold therapy was discontinued immediately. When the drug was omitted as soon as toxic symptoms first appeared, they usually disappeared in a few weeks. The use of gold was then resumed cautiously.

Forty-two per cent of the patients developed some signs of toxicity. This is higher than others reported but it includes even those with mildest toxic symptoms.

Some patients that received the gold treatment for more than a year developed toxic symptoms several times and treatment was discontinued temporarily each time they appeared. If the same dosage was used, toxic symptoms occurred as a rule when the same total amount had been injected, provided the period of rest was sufficient to allow elimination of the gold previously given. If the rest period was insufficient to eliminate it, then toxic symptoms would occur before the same total amount of the drug had been injected.

In analyzing the toxic symptoms that resulted from gold treatment in this study, we observed that low doses produced urticaria, angio-neurotic edema, maculo-papular eruptions and reduction in the platelet count; medium doses, scarlatiniform rash, localized dermatitis, vaginitis, stomatitis, albuminuria, etc.; and large doses produced dermatitis exfoliativa, gastritis, colitis, hepatitis, hiccoughs and encephalitis with vertigo.

**Results.** Over 100 patients were treated with the dosage described. The results compare favorably with those obtained by conventionally larger doses. Fifty-three per cent of the patients were markedly improved with almost complete remission of symptoms: 21% were definitely improved and 12% showed slight improvement. Table 2 illustrates 86% that showed some degree of improvement. 10 cases picked at random from the group considered as having shown marked improvement.

One of the criteria for improvement in arthritis is a reduction in the erythrocyte sedimentation rate. In almost every patient classed as improved there was a reduction in the sedimentation rate, but occasionally a patient that showed subjective and objective signs of improvement, such as increased motion, relief from pain, swelling, stiffness, etc., showed no reduction in the sedimentation rate. There was

definite improvement in a number of instances where toxic reactions developed, even though the total amount of gold salts administered was small.

TABLE 2.—TEN CASES, PICKED AT RANDOM, OF THE 57 THAT SHOWED MARKED IMPROVEMENT AFTER TREATMENT WITH GOLD THIOGLUCOSE

Sex	wly. dose (mg.)	Total dose (mg.)	Interval (Wks.)	R.B.C. (million)		W.B.C.		Platelet count		Sed. rate (mm.)	
				Before	After	Before	After	Before	After	Before	After
F	19	494	26	3.77	7,550	10,200	240,000	190,000	64	15	
F	18	292	14	3.70	6,300	8,250	341,000	205,000	62	12	
M	20	400	20	5.21	12,650	20,000	175,000	232,000	82	14	
M	28	392	14	5.10	4.02	10,000	9,650	260,000	275,000	105	45
M	21	630	30	4.48	4.03	8,400	9,850	208,000	197,000	28	4
F	25	400	16	4.20	3.99	9,700	8,400	309,000	256,000	36	6
F	18	360	20	3.88	3.99	8,400	5,700	218,000	208,000	85	25
F	25	600	24	3.54	4.33	6,300	9,400	219,000	230,000	110	35
F	22	440	20	4.43	4.00	9,400	10,000	190,000	206,000	84	20
F	29	435	15	5.10	4.60	11,200	6,100	250,000	190,000	74	14

The improvement noted was usually so definite both to the physician and the patient that there was no doubt as to the results even though the doses employed were far smaller than conventional ones. There was the additional advantage that the dangers associated with large doses were avoided.

**Discussion.** The results obtained with small doses of gold thioglucose warrant further study of the subject. The use of these small doses enables the physician to detect patients who are hypersensitive to the gold preparation before large total doses have been given, prevents the accumulation of large amounts, and eliminates most instances of serious toxicity which are the constant worry of physicians administering the drug. We did not have any instances of serious toxicity with the small doses used. However, use of even these small doses is not entirely devoid of danger and the physician should be constantly alert for any symptoms of toxicity. Toxic symptoms occurred frequently in the present series but close observation and immediate omission of gold therapy as soon as they appeared usually resulted in only mild and temporary reactions, probably because a smaller amount of gold had been stored in the system and less time was required to eliminate it.

If, as Freyberg showed, less than 25% of the weekly dose of gold is eliminated during the period of treatment, the question arises as to whether it is necessary to store the remaining 75% which appears to be the cause of prolonged toxic symptoms. Our experience indicates that this is unnecessary.

Investigators of gold therapy are well aware of the relapses that occur when the drug is omitted. In the earlier cases of the present series, severe relapses were sometimes observed 1 month after gold therapy had been discontinued in patients who had been obtaining excellent results. With the small dose method proposed in this paper, gold salts therapy can safely be continued for a long time. This method was followed in the latter part of this study and the number and severity of relapses were greatly reduced.

The results obtained by us in 100 cases are comparable with those obtained with conventional doses but there were fewer serious toxic reactions. With conventional doses the toxicity is so great that the method frequently is abandoned. It certainly is dangerous in the hands of inexperienced investigators.

**Conclusions.** 1. It is possible to detect patients who are sensitive to gold therapy in the treatment of rheumatoid arthritis by using much smaller doses than are usually employed.

2. By increasing the dose only as it is tolerated, the number and severity of toxic reactions are markedly reduced.

3. The favorable results (86%) obtained with these small doses are comparable with those reported by others with much larger doses.

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# PROGRESS OF MEDICAL SCIENCE

OTO-RHINO-LARYNGOLOGY

UNDER THE CHARGE OF  
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## VASOMOTOR RHINITIS

BY NOAH D. FABRICANT, M.D.

VASOMOTOR rhinitis is a term currently employed to describe a triad of nasal symptoms: sneezing, nasal obstruction, and a watery or mucoid type of discharge. Occasionally, there may be present such associated symptoms as itching and loss of the sense of smell. The terms vasomotor rhinitis and hyperesthetic rhinitis, according to the more recent literature, are just as fashionable as allergic rhinitis and nasal allergy, this despite the fact that at one time the older literature spoke of paroxysmal rhinorrhea, turgescient rhinorrhea, nasal hydropnea, perennial hay fever, nasal neurosis, nervous coryza, spasmodic coryza, or intermittent neurotic catarrh.

The symptoms of vasomotor rhinitis vary considerably in different individuals both as to degree and duration. Any one of the nasal symptoms may dominate the clinical picture and any one, in turn, may be submerged. Sneezing occurs in paroxysms and is accompanied by a watery, alkaline, irritating type of nasal discharge. In the presence of an associated secondary infection, the nasal discharge may become mucopurulent. Itching of the nose may precede sneezing or be dissociated from it. Not infrequently, itching involves other structures—the eyes, mouth, pharynx, and occasionally the larynx. Nasal obstruction is usually pronounced and incurs considerable discomfort. The nasal mucous membranes usually are pale and appear swollen and “water-logged.” More rarely, the mucous membrane will show a pinkish color or even slight congestion and redness.

There has been a pronounced tendency among allergists to regard the vast majority of cases of vasomotor rhinitis as being based on an allergic origin. This point of view has gained many adherents in rhinologic circles. Nevertheless, some rhinologists, in the light of negative skin tests and by the apparent absence of the usual inhalant, protein and food factors, consider numerous cases of vasomotor rhinitis to be of non-allergic origin. Allergic Aspects. Since there is some vagueness in the term “vasomotor rhinitis,” Urbach<sup>30</sup> has proposed a specific classification. He offers the term “rhinopathy” to cover all the conditions known as rhinitis and, in turn, differentiates this as allergic rhinopathy when the cause is specific

allergy, and pathergic rhinopathy when the cause is a non-specific hyper-sensitivity. Allergens to be considered under allergic rhinopathy are: exogenous substances, or those exerting an influence on the nasal mucous membrane; foods or drugs taken by mouth; and endogenous substances, such as products of the endocrine glands, of metabolism or of bacteria in the intestines. Pathergic rhinopathy can be the result of an underlying general or nasal infection, of a local irritation, of psychic disturbances, and also of a non-specific broadening of a hypersensitivity which was formerly specifically allergic. Included under pathergic rhinopathy are the paroxysmal attacks of sneezing with subsequent swelling of the nasal mucous membrane, following exposure to sudden changes in temperature; attacks of sneezing attributable to hypersensitivity to cold or heat; and sneezing brought on by looking into bright sunlight. On the basis of 74 cases, Urbach found that in 54 individuals the symptoms were caused by a variety of non-specific factors; in about two-thirds of the remaining cases, they were specifically allergic; in one-sixth, they were due to endocrine disturbances, and in one-sixth to intestinal disturbances. He believes there is no standard method of treatment. Whenever possible, each patient should be treated etiologically. Operations are usually without lasting effect, and occasionally they are even harmful. Treatment of allergic rhinopathy depends on elimination or desensitization, and treatment of pathergic rhinopathy consists in an attempt to lower the hypersensitivity of the nasal mucous membrane and simultaneously to increase the patient's resistance.

In a discussion of the allergic aspect of vasomotor rhinitis, Gelfand<sup>12</sup> states that three guides to successful management are complete history, clinical observation and exhaustive tests. A history should include the patient's own description of home and occupational environment, of home, business and social contacts, of cosmetics and clothing used, hobbies, sports, and other activities. The food intake should be checked by food diary or elimination diet. Intradermal tests should cover all possible materials to which the patient may be allergic. After employment of all possible irritants and in various concentrations, the patient can be considered cutaneously insensitive to that particular material only if he fails to react to the strongest extract. It is believed necessary to use every kind of allergen to which the patient has shown sensitivity, even when his contacts with some have been casual or infrequent. Intensive therapy consists of rapidly working up to a dose by increasing the frequency of treatments to twice or three times a week and then continuing with the maximum dose at short intervals. Analysis of 52 cases of perennial vasomotor rhinitis revealed an allergic origin in 89%. Most patients showed multiple sensitivities, but, with few exceptions, vasomotor rhinitis was the sole clinical manifestation; only a few had hay fever, asthma and urticaria. Dust sensitivity was present in 95%, and sensitivity to occupational allergens other than dust was demonstrated in 41%. These allergens included animal fur, dyes and chemicals used by furriers, feathers, wool, cotton, kapok, wheat, rye, pyrethrum, and odor of paint. Housewives showed sensitivity to dust,orris root, feathers, tobacco, perfumes, and animal epithelium. Treatment consisted of injecting or avoiding all specific allergens that could be demonstrated; in some patients both measures were utilized. Of the 52 patients, 24 improved, 6 were relieved, 10 had fair results, and 12 were unimproved or referred elsewhere. Some investigators<sup>13,14,15</sup> have identified molds, yeasts, rusts and smuts, insect emanations, and other inhalants as etiologic factors in perennial vasomotor

rhinitis. Ingested drugs and chemicals have also been implicated. Eyer<sup>1</sup> and Mann<sup>2</sup> studied 181 patients with vasomotor rhinitis due to ingested foods and found that the foods most frequently eaten were the ones most often responsible for sensitization. He advised that the influence of foods and inhalants be investigated before resorting to injections of pollen. Sternberg and Sorrell<sup>3</sup> refer to specific occupations in which vasomotor rhinitis and asthma occur when the potentially sensitive subject comes in direct contact with certain antigens to which he becomes specifically sensitized through direct exposure. They state that such individuals may give positive cutaneous reactions which are not due to chemicals or drugs. After studying 45 patients with vasomotor rhinitis, Shahan<sup>4</sup> advances the following recommendations: removal of offensive inhalants and foods from the patient's environment; employment of immunization or hypersensitization when necessary; provision of a balanced diet after offensive foods have been eliminated; removal of foci of infection; palliative medical treatment; and change of environment, when possible. Cooke<sup>5</sup> also claims that the treatment of vasomotor rhinitis consists of recognition and avoidance of the cause, and immunization, or the attempt to develop a protective antibody. Some proteins in ragweed pollen cause marked skin reactions but stimulate the protective mechanism only slightly. If after injection there are symptoms of a general reaction, 0.5 cc. epinephrine (1 to 1000) should be given hypodermically and the tourniquet used promptly. Little can be done with drugs to allay the symptoms of hay fever, and cocaine sprays should never be employed since the habit is easily formed. A 1% ephedrine spray may give temporary relief but should not be employed over a long period of time or too frequently.

In a discussion of the significance of the nose in allergic states, Frackelton<sup>6</sup> declares that an allergic state may be prolonged as a result of the locking up of residual allergens within the sinuses or being trapped beneath the turbinates. Swelling of these bodies, resulting in prolonged contact with the septum, may cause the allergen to penetrate to the submucosa and produce sensitivity of the underlying perosteum and bone. He believes that post-nasal irrigation with isotonic solution of sodium chloride enables the fluid to reach the nasal spaces not accessible to anterior irrigation, with removal of residual allergens. The same principle applies to the maxillary sinus, which should be irrigated after an attack of nasal allergy or after the hay fever season. Frackelton also recommends "allergic surgery," whenever indicated, so as to relieve the tissues from pressure due to anatomic or pathologic changes. By "allergic surgery" is meant the correction of a deviated septum, removal of spurs, trimming the hyperplastic turbinates, or amputation of the anterior end of the middle turbinate. It is claimed that the freeing of the intranasal structures from the possibility of prolonged contact of mucous surfaces prevents intramucosal penetration, and when this is done in conjunction with the irrigation the possibility of absorption of allergens, as well as of secondary infective agents, is prevented and the remote effects so often seen in allergic patients are guarded against. King and King<sup>7</sup> write that despite every measure adopted and every effort put forth by the allergist and the rhinologist, there are patients whose disease is refractory to treatment. They review and analyze their experiences with 9 patients, all having the same nasal symptoms, their reactions to cutaneous tests being negative, and their disease refractory to treatment. The following recommendations are advanced: determine the skin reactions as soon as possible; control the environment; try diets strictly free from milk and wheat over extended



periods. If there is no response, bacterial allergy should be considered. Cultures of the nose and sinuses can be made and the administration of remedies such as histaminase, thyroid and estrogens, with chemotherapy given in selected cases. The authors believe that good results will come in the vast majority of cases only through careful, painstaking effort with each individual patient.

Riccielli<sup>24</sup> discusses the influence of season and climate in relationship to the various manifestations of nasal allergy. He asserts that cosmic and meteorologic conditions have a deep influence on physiobiologic processes through the changes in temperature, variations in the amount of actinic rays, and the degree of electricity in the air. All these factors are more or less rhythmic in their changes, and therefore it may be expected that allergic persons will react correspondingly. Spring is the season when hyperergic reactions are most manifest and at this time the inorganic phosphorus and calcium contents of the blood are lowest. Therefore, a study of mineral metabolism is important. Luschka<sup>25</sup> attributes the allergic tendency to a hereditary hypersensitivity of the vegetative nervous system and a disturbance in the neurovegetative balance whereby the autonomic nervous apparatus affects the local reaction in tissues to various irritants. He feels that in cases of such hypersensitivity there frequently may be an additional psychic factor which enhances the reaction. He cites the various stigmas so commonly existing in such cases and agrees that the presence of eosinophilia in the secretions, tissues and blood is a valuable diagnostic sign. Jaffe<sup>26</sup> reports his observations on vasomotor rhinitis in the tropics and states that in some cases an allergic origin has been demonstrated while in others such an origin is questionable. The prognosis is rarely serious, but a complete cure is difficult to obtain. One hundred and thirty patients with vasomotor rhinitis were treated in the republic of Honduras in Central America. Vasomotor rhinitis was found to be quite common in this climate. There was a great deal of dust, especially in the cities, and it was felt that this was a factor. The patients were treated by cauterization of the mucous membrane with a concentrated solution of trichloroacetic acid. The author warns against unnecessary operations, and suggests methods of conservative therapy which have been found to be satisfactory. Glover<sup>27</sup> finds that an allergic nose must sooner or later be subject to infection because of the impairment of ventilation and ciliary action caused by the allergic swelling of the tissues. It is therefore important to treat the infection actively as well as to employ measures capable of overcoming the allergic state. Farmer and Kaufman<sup>28</sup> have employed histamine in the treatment of perennial and seasonal allergic rhinitis. The histamine was given by subcutaneous injection, and the injections were increased each time by 50% if well tolerated. Among 41 cases of perennial rhinitis the result was reported good in 25, fair in 10, and poor in 6. Successful results were obtained in 26 of 33 patients with perennial vasomotor rhinitis by Gant, Savignac and Hochwald<sup>29</sup> after each individual dosage of histamine (1 to 1000) dilution had been established.

**Non-allergic Aspects.** According to Mohn<sup>30</sup> vasomotor rhinitis and associated conditions have a definite incidence during pregnancy. He describes the cases of 8 pregnant women who experienced a severe degree of nasal blocking and congestion during pregnancy; in the non-pregnant state none of these women had ever experienced such difficulty. Twelve other pregnant women experienced severe nasal blockage, but these

patients had a history of previous transient attacks of hay fever and nasal blocking in the non-pregnant state. In 9 patients with purulent sinusitis the infection was more protracted and the mucous membrane more swollen than is usually encountered. They showed no response to customary therapy, but the condition cleared quickly after delivery in all instances. From both a theoretic and clinical standpoint, allergy did not seem to be the predominant etiologic factor. The incidence of vasomotor rhinitis during pregnancy appears to be parallel and to be caused by the amount of estrogen produced in the body. Characteristically, the condition disappears spontaneously after delivery. Fowler<sup>8</sup> describes a case of unilateral vasomotor rhinitis due to interference with the cervical sympathetic system. This case suggests to him that experimental surgery might uncover the fundamental mechanism of vasomotor rhinitis. If one can produce vasomotor rhinitis at will, one should be a long way toward understanding allergic diseases more thoroughly. Animal surgery may localize the part of the sympathetic or the parasympathetic nervous system which needs to be attacked for prevention or cure. Citing the report of the Asthma Research Council after 5 years of investigation, Stovin<sup>28</sup> states that the reasons for dissatisfaction with the therapy of vasomotor rhinitis are to be found in too much dependence on desensitization and not enough on body chemistry. There may be an unrecognized imbalance, for example, between the cations of sodium and those of potassium. In contrast to the diminishing effectiveness of epinephrine is the sustained and often increasing effectiveness of supportive therapy. Stovin believes that the use of diet rich in protein and acid ash and low in sodium chloride, combined with the administration of potassium iodide and bicarbonate, has proved effective. A similar diet, as for bronchial asthma, combined with the administration of the chloride salt of potassium has given excellent results in the general treatment of vasomotor rhinitis.

**Nasal Medication.** According to Houser,<sup>17</sup> anti-allergic therapy often fails to secure satisfactory results, although there are enough successes, in full or in part, to justify the continuation of allergic studies and treatment. There are certain measures worth trying in the attempt to relieve individuals who in spite of all efforts remain wrecked and partly incapacitated with blocked and discharging noses. The measures most likely to secure results all have as their goal a desensitization of a considerable portion of the nasal mucous membrane. Electrocoagulation, the galvano-cautery, the injections of solutions of alcohol, guinine and urea, and sodium morrhuate, the application of a chromic acid bead or a 30% solution of phenol and trichloroacetic acid have been reported as measures improving or freeing the patient of symptoms for a period of time varying from a few weeks to many months. In spite of the possibility of securing results by these procedures, the nasal mucous membrane should not be subjected to these relatively harsh measures. Houser states, unless anti-allergic therapy has failed. The use of a watery paste of resorcinol has been suggested by Levy.<sup>21</sup>

The administration of sclerosing preparations into the submucosa has a long list of ardent devotees. In general, these agents include hypertonic saline solution, glycerin, sodium salicylate, quinine lactate, alcohol, guinine and urea hydrochloride, a 5% solution of calcium sodium lactate, and sodium morrhuate. Vail<sup>21</sup> has favored the injection of alcohol into the inferior turbinate and septum, while Ruskin<sup>22</sup> and Walsh<sup>23</sup> have injected alcohol into the nasal ganglion. Fishof<sup>7</sup> and Thacker and Houser<sup>29</sup> have reported that vasomotor rhinitis is relieved by submucosal injections of a

5% solution of sodium morrhuate. They believe that the use of this agent does not impair the physiology of the nasal mucous membrane and that, clinically, no deleterious effects are detected. There is comparatively little pain or discomfort after the injection, and most patients require but 1 or 2 injections of 0.5 cc. of the 5% sodium morrhuate solution in one or both inferior turbinates. Associated symptoms, such as headache, neuralgia of the sphenopalatine ganglion, and post-nasal dripping were markedly diminished or cured. In the experience of Fox,<sup>9</sup> the use of sodium psyllate solution (Sylnasol) in more than 200 cases of chronic vasomotor rhinitis by injection is highly successful.

The status of zinc ionization (iontophoresis) with respect to vasomotor rhinitis still remains a subject affording considerable difference of opinion between the allergist and the rhinologist and between one rhinologist and another. Those who have had much experience with the procedure in select cases still advocate its use; those who dispute its value are increasingly antagonistic to its employment. While a good part of the controversy settles on the question of how much damage actually is done to the nasal mucous membrane following zinc ionization, it has been pointed out that electrocautery, certain drugs and chemicals, Roentgen rays and radium often produce much greater damage to the nasal mucous membrane than does ionization. Kuhn and Linton<sup>20</sup> discuss their limited experiences with the use of ionization. In a few cases where there was no improvement with hyposensitization and in cases where the symptoms of seasonal allergy were well advanced at the first examination, ionization has been found satisfactory in about 50% of the cases. The authors did not encounter the destructive and atrophic changes attributed by some writers to ionization. In nasal cases in which no allergen could be found, and the patient is proved to have a low basal metabolic rate, small doses of thyroid have relieved the nasal symptoms completely. Glass<sup>21</sup> employed 1 to 2000 and reported excellent results in some instances.

The use of liquid nasal medicaments for the relief of nasal discomfort in vasomotor rhinitis is indicated, even though the relief is often temporary; for even a measure of relief is welcomed by distressed and distracted patients. At one time solutions of ephedrine administered by nasal spray or medicine dropper had considerable vogue. With the advent of ephedrine and synthetic ephedrine-like preparations, the employment of ephedrine has, for the most part, fallen by the wayside, for when the effects of the latter wear off nasal symptoms may return in a more exaggerated form. Ephedrine, therefore, is not a suitable agent for local application in the nasal cavity. Ephedrine in 1% physiologic saline, or one of the synthetic preparations, is more effective and is generally used for relief. Occasionally, undesirable side effects are produced as a result of absorption through the mucous membrane, but as a general rule this does not take place.

Hansel<sup>18,19</sup> asserts that in the field of allergy the cytologic content of nasal and sinus secretions may be considered an approximate index of pathologic processes occurring in the tissues, and that the cytologic observations give important diagnostic information. In allergy, it is particularly significant that when the pH of these secretions falls on the acid side, there is a complete disappearance of the eosinophiles. When the pH returns to the alkaline side, there is a return of the eosinophiles. Fabricant<sup>22</sup> tested the pH of nasal secretions *in situ* in cases of allergic rhinitis and found these secretions to be alkaline during the active stages

of the allergic rhinitis. The nasal pH descended to the neutral point and then to a slightly acid status when clinical improvement was felt by the patient. Whenever the symptoms became aggravated, a return to an alkaline status was detected. From the point of view of applied nasal therapeutics, Fabricant suggests that during the more active stages of allergic rhinitis the employment of a nasal vasoconstrictor which can lower the abnormal alkaline nasal status to a normal, slightly acid status—a pH level between approximately 5.5 and 6.5—may perform a valuable function.

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## DERMATOLOGY AND SYPHILOLOGY

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## THE "LUPUS ERYTHEMATOSUS" CONCEPT: AN ATTEMPT AT INTEGRATION

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The manner in which the synthetic creation of entities from symptomatology may occasionally leave the specialist out on a limb etiologically is well illustrated by the complex misnamed "lupus erythematosus." A syndrome of multiform character, with lesions ranging from the insignificant local cutaneous to the promptly fatal polystystructurally systemic;

originally described by the dermatologist Hebra<sup>84</sup> as part of the seborrheic-infective complex and now in fair way to be swallowed by the internist as a disease of the vascular system, lupus erythematosus has at best only a distant relation to the cutaneous tuberculous processes suggested by its conventional name. The literature reveals a variegated patchwork of opinion as to cause, slowly working into a pattern that will combine a central etiologic conception with a wide range of peripheral manifestations. This review aims to outline such a conception.

When the dermatologist wishes to hypothesize on variegated peripheral manifestations, he turns to the vascular mechanism as providing the most immediately eligible network for dissemination; and when local particular noxae, like microorganisms in lesions, cannot be found, he tends these days to turn to infection allergy in its various expressions, including the Samarrelli-Shwartzman<sup>73, 78</sup> phenomenon to explain the clinical and histologic characteristics of the dermatosis. In dermatologic terminology as applied to lupus erythematosus this means an effort to combine the multiform erythema syndromes with the "id" concept in such a way as to account for the multiplicity of clinical manifestations that appear as variants upon the erythematosus lupus base. So wide is the range of variation in symptoms and course that the theory must account at one sweep for a life-time of benign follicular keratitic patches below the malar prominences, in 1 case, the livid purple erythema of the Kaposi<sup>10</sup> facial eruption in another; and the occasionally almost lesionless skin of the young woman dying with nephritis and pneumonia of acute disseminated lupus erythematosus in a third. 1.5, 7.7, 10.11, 13.22, 23.25, 26.29, 31.38, 39.41, 41.42, 44.45, 45.55, 56.59, 64.69, 70.71, 74.81, 84.85, 86.90.

**Nomenclature and Classification.** Ambrosetti<sup>1</sup> has collected most of the classification that has developed about the clinical picture from Hebra's<sup>84</sup> "seborrhoea congestiva" to Kerl's amplification of Bhrmann and Ralkenstein's<sup>82</sup> classification (of which Urbach and Thomas's<sup>86</sup> is a modification), which includes 6 types based largely on the acuteness and dissemination of the process. It seems best to stress here the localized discoid and the disseminated multiform types of lesions, as the two main categories, from which sub-varieties of acute and subacute discoid and localized and disseminated discoid can be made up, as well as acute and subacute multiform varieties. The discoid and the multiform types may interweave, disclosing 1 or 2 discoid chronic lesions in the scalp of a patient dying of acute multiform or disseminated erythematosus lupus; or a discoid chronic localized type of the disease of years' standing suddenly passing over into an acute multiform disseminate type with rapidly fatal outcome. This confusing polyphase character, heterogeneity of symptoms, and interweaving of types can, it seems to us, best be resolved by setting the picture over against two groups of dermatologic lesions now well known to be among the expressions of infection-allergy; the cutaneous *follicular* "ids" (trichophytids, tuberculids, mycotids, staphylococoids, streptococoids, etc.) and the multiform erythematosus as expressions of vascular injury and allergy. **Application of the "Id" Conception.** The elementary lesion of chronic discoid erythematosus lupus is a follicular inflammation with atrophy. It differs essentially from the follicular lesions of other types of "ids" such as trichophytids, follicular tuberculids (lichen scrofulosorum) only in that the plug or "prop" is more conspicuous than the papule, and in the sequelae of atrophy, which rarely follow the involution of other follicular "ids." That the histologic picture presented by the follicular lesions of erythematosus lupus is that of allergic inflammation is supported by such

studies as those of Baccaredda,<sup>4</sup> who regards the cellular picture as comparable to that found in tuberculids and "microbids." The homologues with allergic inflammation are also stressed by Rost.<sup>72</sup> Fibroid degeneration of the collagen, which may lead to the atrophy and scar in discoid lupus erythematosus, were experimentally produced by Gerlach<sup>78</sup> and Klinge<sup>76</sup> in another field—that of rheumatic fever—and interpreted as must, however, recognize that there is not as yet a general acceptance of colloidal degeneration as a sign of allergic reaction. Klemperer, Pollack and Baehr<sup>74</sup> have in fact vigorously opposed it in their description of "diffuse collagen disease" as a pathogenic entity. As a theoretical basis for classifying the follicular lesion of discoid erythematosus lupus as an "id" or allergic reaction to an infection, the allergic inflammation concept of Rost and others has, then, it would seem, some merit. Using such an interpretation, one might link together through the occurrence of similar "allergic" histologic change in other than skin tissues and in vessel walls, renal glomeruli, and so forth, the chronic discoid form of erythematosus lupus and its clinical team-mate, acute disseminate erythematosus lupus. The range of infection-allergic inflammatory disease might even be made to include the Libman-Sacks<sup>75</sup> syndrome, the sclerodermatomyositis complex, and periarthritis nodosa (Banks<sup>79</sup>).

If the discoid type of erythematosus lupus be then thought of as a follicular "id" or infection-allergic reaction in the skin proper, a variety of scattered considerations becomes to some extent reconcilable with clinical experience. The folliculo-allergic reaction as it might be designated, in discoid lupus erythematosus, commonly occurs over the most light-exposed parts of the face. Light is a well-recognized physical allergen. A variety of other influences may still further enhance the allergic effect. The sites of involvement are, for example, also those of the flush area of the face and head as well as those of light exposure; and a rich congestive vascular background, as in the Meitzner-Auer<sup>3,57</sup> conception of local allergic response, contributes markedly to allergic reaction. Thus circulating allergens brought from distant points to a congested area might exacerbate a discoid process in lupus erythematosus. Emotion as a vasodilator might then contribute to persistence and relapse in lupus erythematosus as observed by Callaway and Stokes.<sup>13</sup> Circulating light sensitizers (porphyrins) would be brought in increasing amounts to congested areas specially bombarded by light rays.<sup>8,51,88a</sup> Light and cold as physical allergens known to produce exacerbations of erythematosus lupus would be acting conceivably not only on their own allergic merits but also as powerful local insults to stimulate the injurious effect of an underlying infection-allergy. The commonly observed clinical association of discoid erythematosus lupus of the face with the seborrhoeic-infective process in the scalp gives a clue to a possible source of infection-allergy which may apply also in the more obscure and complex picture of the Senear-Usher<sup>90</sup> syndrome. Discoid lupus erythematosus can be hypothetically accounted for then as follicular (seborrhoeic) infection plus allergic reaction to that infection (yeasts and staphylococci). The dissemination of a discoid or follicular erythematosus lupus (a lupus erythematosid?) with the appearance of desquamative patches with follicular atrophy over other parts of the body, is simply an extension of the follicular "id" beyond its conventional locus, under conditions affecting the general allergic state of the individual. His allergic base can be broadened by

one or another influence, bringing on such exacerbation and generalization. It may even be by the administration of a sulfonamide, which in accord with Barber's<sup>5</sup> conception causes exacerbation and dissemination by flaring a concealed systemic focus of infection (liberation of streptococcal toxin), thus modifying the local follicular and the general skin allergic substrata. To none of these "id"-like processes, which are allergic manifestations in the skin itself, need grave prognostic significance usually attach—the benign prognosis of the disseminating discoid type of the disease is well known. *On the other hand*, in the hypersusceptible person—the infection-allergic type of individual, let us say—the epidermo-allergic or folliculo-allergic type of reaction that underlies the chronic discoid process may at a suitable provocation pass over (though only occasionally) into the field of vasculo-allergic manifestations and assume the far graver characteristics of the so-called acute disseminating type of the disease (*cf.* Fox, 1943<sup>24</sup>). **The Vasculo-allergic Manifestations in Lupus Erythematosus.** As the discoid type of lesion is expressive of local cutaneous infection-allergy of the follicular inflammatory type with atrophy, so the acute disseminating type is the clinical type of lupus erythematosus with multiform disseminated cutaneous and systemic lesions resulting from allergic inflammation of the vascular system. In order to conceive of acute disseminating lupus erythematosus as an erythema multiform, it is necessary to expand one's clinical conception of the multiform erythematosus to a degree not always acceptable to dermatologists.<sup>41a</sup> The multiform erythematos are, however, generally conceded to include acute and chronic types of which erythema figuratum persans, erythema elevatum diutinum, sarcoid-like infiltrations and even granuloma annulare are well recognized or extreme and controversial variants. In the chronic types of erythema multiform the experienced observer will find examples of the atrophic changes of erythematous lupus side by side with the edematous wheals, the purple plaques, and circinate and gyrate figures of erythema multiform. The "deep" types of indurative erythematosus lupus are approaches to the multiform erythema group of erythematosus lupus manifestations. The Kaposi<sup>40</sup> *erysipelas persans faciei* is morphologically a chronic erythema multiform early recognized as one of the forms of disseminating erythematosus lupus. The pellagroid type of lesion, the acute edema and exfoliation, and even focal necrosis of the skin of the face representing acute light injury in a rapidly fatal erythematosus lupus is obviously a different process from the local follicular "id" of the discoid lesions but it is nonetheless an allergic response. Such reactions represent, perhaps, localized acute vascular injury, occurring when a circulating photosensitizing allergen meets the effective wave length of actinic energy. When we come to the more extensive processes, the multiform nodose and plaque types of lesions, the infiltrations of skin and subcutaneous tissue,<sup>7b,38</sup> the infiltrative livedo and Bazin-like tubercloid lesions that the infection-allergic nature and vascular distribution of the process or agent is also strongly suggested. For this group of widely disseminated multiform manifestations in the erythematosus lupus complex we would suggest the designation "acute or chronic vasculo-allergic type," using "chronic" rather than "acute" in certain cases, in deference to the view of such observers as Rose and Pillsbury<sup>71</sup> that disseminating erythematosus lupus is a long-standing disease of the vascular system before it comes to acute exacerbative manifestations in the skin.

The vasculo-allergic type of "lupus erythematosus" then, may be preponderantly local and cutaneous in its manifestations, or preponder-

antly systemic (fever, lymphadenopathy, leukopenia, absolute or relative, with or without thrombocytopenia, and with demonstrable vascular lesions of the eyes, kidney and endocardium). It may be acute or chronic or both. It may be combined with follicular "id" lesions of discoid type and distribution, or not. The systemic vasculo-allergic lesions may come to notice first and dominate the picture to exitus. But we suspect as dermatologists that the insistence of certain authors and clinicians that a complete absence of skin lesions is not uncommon, may be due to inadequate search for or non-recognition of the cutaneous ones. Certainly we believe that a diagnosis of disseminate erythematous lupus sine erythematous lupus, in the broad interpretation of multiform erythematous lesions, must be made with caution.

**The Infection-Allergic Mechanism in Lupus Erythematosus.** Such emphasis on allergy of infection, cutaneous (follicular or epidermal) and vascular, as capable of welding into a comprehensible unity the disparate and confusing interplay of lesions included among the captions "discoid" and "disseminate," "acute," "subacute" and "chronic," now requires an accounting. It must be confessed that the clinical case for infection allergy is not massively *pro*, though there is also little *con*. The importance of infection allergy in the lupus erythematosus complex is supported by (a) the extreme, even fatal reactivity of disseminate types, to tuberculo-toxin; (b) the recognized danger of stirring up a focus of infection (especially dental) in disseminate cases; (c) most recently by experiences with the sulfonamides, which appear to help some cases by subduing an infection focus, or, in reverse, make others worse as in Barber's observation<sup>6</sup> by activating or stirring up a focal infection.

As to the nature of the infection to which allergy develops or exists, no absolute decision can be had at this time. There is sharp disagreement on the presence of tuberculous infection in individuals coming to autopsy with erythematous lupus. Many authors are still aligned on the tuberculous side, while the more recent trend is toward the non-tuberculous nature of lupus erythematosus. The data on this subject in the literature from 1871 to 1942 is tabulated by Ambrosetti.<sup>1</sup> A similar controversy has persisted over the years, it will be recalled, in regard to erythema nodosum, which has finally been stilled by the compromise that in some countries it is often tuberculous, in others rarely so. In other words, there is a tuberculously-backgrounded form of the non-specific erythema nodosum picture. Why can there not be such in lupus erythematosus disseminatus? Streptococcal infection has on the whole made a much better case for itself. Among the authors supporting the view that bacterid (streptococcal) foci (toxemia) are etiologically important in the background of the disease complex are Kell,<sup>11a</sup> L. W. Shaffer<sup>7</sup> and Barber.<sup>6</sup> It is, however, more or less accepted that the organisms obtained from the blood stream in occasional cases (tubercle bacillus, *B. alkaligenes fecalis*, *Staph. aureus*, pneumococcus, *Strep. an-hemolyticus* and *hemolyticus*, *Strep. viridans*) are coincidental,<sup>11a, 10</sup> or are terminal bacterial invasion.<sup>47</sup> A possible virus (ultramicroscopic agent) etiology has been suggested by Belote.<sup>10</sup> No experimental work as yet has accomplished the reproduction of the clinical picture of erythematous lupus in any of its forms, in animals. On the allergy side of the problem clinically, there may be cited the extreme, even rapidly fatal reactivity to certain infective agents or toxins above mentioned. The occurrence of leukopenia as an almost unvarying accompaniment of the disseminate and acute types of erythematous lupus, is perhaps suggestive of an allergic



assault on the bone marrow, though a number of observations attest the normality of the bone marrow in some of the cases in which it was studied. Thus far, the subject has been inadequately examined from the standpoint of the allergic background, familial and personal, in either the acute or of the chronic types. As an allergy of infection, it is of course possible that the yield of such inquiry would be small when related to allergic reactivity in general. The *photosensitibility phenomena* in lupus erythematosus furnish some of the strongest evidence for the allergic factor, yet photosensitivity seems to play no part in many cases and the incidence is not statistically overwhelming in countries or at seasons in which sunlight is particularly abundant. That porphyrias are merely incidentally present in the stools, blood or urine is contended by Goeckerman, Osterberg and Sheard.<sup>32</sup> The more recent work tending to establish certain types of intestinal organisms as responsible for the formation of light-sensitizing porphyrins, however, suggests a mechanism by which a bacterial focus (the intestinal tract is rarely investigated as such in studies of focal infection in lupus erythematosus) can provide an agent for an allergic injury to the skin which "sets off" the disastrous train of explosive reactions that destroys the patient with acute disseminate lupus erythematosus.

**Treatment Conceptions and the Foregoing Theorization.** It must be confessed that the contribution of a concept of infection-allergy to the treatment of lupus erythematosus is largely negative. It warns (1) that most lupus erythematosus is likely to become a grave problem if recognized too late—after the allergic changes in the vascular bed have become established in vital structures and grave damage is done. (2) It warns that disseminate cases are dynamic—must not be tested for tuberculousity with tuberculin, must not have infected teeth rashly extracted, or in periods of exacerbation. (3) It warns that sulfonamides must be used with the utmost caution, though helpful in some cases.<sup>2,6,9,15,30,35,36,37,49,53,65,80,88,92,93</sup> (4) The appearance of multiform erythematosus as distinguished from discoid (follicular) lesions is of grave prognostic significance as suggesting involvement of the vascular bed, and a circulating "toxin" or allergen. (5) Leukopenia is an unfavorable sign, and thrombocytopenia even more so. (6) Fever, serous exudation, pulmonary symptoms and lymphatic enlargement, and especially albuminuria and impaired renal function are suggestive of the disseminate type and the existence of a deep as distinguished from a superficial process and injury.

**The Early Diagnosis of Vascular-Allergic and Disseminative Tendencies.** How shall lupus erythematosus be recognized, suspected, or forestalled before it is clearly lupus erythematosus? (1) The preponderance of women, in the ages from puberty to menopause; (2) a history of light sensitiveness; (3) pallor and asthenic habits with "fever of unexplained origin" (which is one of the synonyms for acute disseminate lupus erythematosus); (4) leukopenia, persistent and unexplained, especially during febrile episodes; (5) puffiness and persistent lividity of the lower eyelids; (6) thinning hair with no apparent local cause; (7) arthritoid manifestations in which the pain but not the fever responds to salicylates; (8) petechial eruptions; (9) slight albuminuria; (10) pleural, pericardial and endocardial signs with persistently negative blood cultures; these are, in various combinations, of considerable significance and the Reifsteins<sup>59</sup> and Rose and Pillsbury<sup>70</sup> even express willingness to make a diagnosis on these signs before the development of characteristic lesions.

**Treatment.** When once the process can be recognized, what can be done? It is not intended here to go into the therapy of the localized

disoid type. Gold therapy has not proved to be the radical cure that was hoped for, notwithstanding its good effect on many cases. Callaway and Stokes<sup>13</sup> pointed out the frequency of relapse and some of the factors influencing it. The now clearly recognized toxicity of gold is especially serious in cases threatening dissemination and there are no established benefits from its use in such cases.<sup>1,7a,12,20,50,52,53,51,94</sup> Bismuth is rated by Smith<sup>79</sup> and others<sup>1,53,62,75,76,84,89</sup> as equal in effectiveness to gold and materially safer, an experience with which the Reviewers agree. The non-specific quality of the effect is evident from the fact that neosilver arspena-mine for example, other trivalent arsenicals and even pentavalent arsenicals are also effective in some cases in which bismuth fails.<sup>1,19,52,65,87</sup> Other non-specific measures—liver extract, chaulmoogra oil—have been tried.<sup>12,17,43,50,66,67</sup>

In all types of erythematous lupus, the beneficial effect of rest can be observed, but it becomes paramount in those presenting threatening acute or disseminative manifestations. Rost gives it prominence<sup>72</sup> in his discussion before the Strasbourg Reunion. The adjunct of *quinine* internally<sup>18</sup> is as much tradition as anything, but is supported by a number of experienced observers. It should be pushed to at least 30 gr. a day. Its local use is mainly as a light-protective. *Protection from light* must be carried to the point in acute cases of keeping the patient in total darkness for days and weeks in order to tide over an acute light-exacerbative tendency. Nicotinic acid amide (nicotinamide) has also been used presumably with beneficial effects because of actions of light rays.<sup>27,46</sup> In the febrile repeated small transfusions are as useful as the acute disseminate type, a patient over to a period of improvement, but the story of the ultimate outcome is told by figures such as those of the Mayo Clinic Group.<sup>55</sup> The association of the acute disseminate type of erythematous lupus with the years of active menstrual function in women has led to assumptions of endocrine influence on the process. As a summary of the present extremely limited knowledge of the subject we are privileged to quote *in toto* a personal communication from Dr. Edward Rose who has been especially interested in this problem.

"The striking frequency with which the systemic exacerbations of acute disseminated lupus erythematosus exhibit a predilection for women in the active sexual phase of life (?; e., between puberty and the menopause) has led to considerable speculation regarding the possible role of ovarian hormones in the disease. To date, no evidence has been presented which offers proof of any such relationship, but several attempts have been made to treat exacerbated lupus erythematosus in the female by suppressing ovarian function. Contratto and Levine<sup>16</sup> in 1939 reported the use of irradiation therapy to the ovaries in 1 case. Sossman and his associates<sup>\*</sup> have employed this type of treatment in several other cases at the Peter Bent Brigham Hospital in Boston. Cluxton and Krause<sup>14</sup> mentioned irradiation of the ovaries as worthy of trial, in their review of acute disseminated lupus. Rose and Pillsbury<sup>†</sup> have collected 28 cases of acute females; 19 of these were in active menstrual life and 17 were between the ages of 14 and 30. In 1 of their male patients, the urinary excretion of 17-keto-steroids was low and the urinary excretion of pituitary follicle-

\* Sossman, M., in personal communication to Dr. Edward Rose.  
† Rose, E., and Pillsbury, D. M., unpublished data.

stimulating hormone was increased, suggesting hypogonadism with an increased estrogen-androgen ratio. Rose and Pillsbury\* have observed the effects of inhibited ovarian function in 6 women with lupus erythematosus. Two of their patients showed only cutaneous lesions of the face. One of these women gave a history of premenstrual exacerbations of the skin lesions and showed marked improvement after a menopausal dose of Roentgen ray was given. The other patient has shown no marked improvement 10 months after irradiation menopause. One patient with a history of several acute systemic exacerbations has shown marked improvement since entering a natural menopause, while 1 patient whose clinical picture was complicated by probable bilateral apical pulmonary tuberculosis has remained well for 20 months after surgical castration (chronically infected Ralloppian tubes were found and removed at the time of oophorectomy). One patient died of renal failure several months after Roentgen ray castration, the clinical course of her disease apparently being unaffected by the procedure. One patient had had lesions limited to the face with premenstrual exacerbations for several years prior to a severe systemic exacerbation. Following irradiation menopause, the acute visceral phenomena subsided, leaving the patient with evidence of rather widespread vascular damage including extensive degeneration of the retinal vessels. This patient died 3 months after irradiation was given. These data quite obviously do not prove that castration in the female, whether accomplished surgically or by irradiation, is a therapeutic agent of value in the treatment of acute disseminated lupus erythematosus. However, in view of the extremely grave prognosis of the acute exacerbated form of this disease, it would appear justifiable to undertake further study of possible relationships between gonadal hormones and lupus erythematosus and to investigate the possible usefulness of castration in female patients more extensively."

By what mechanism the ovarian pituitary hormonal complex may be conceived to influence the infection-allergic picture of lupus erythematosus must be a matter of speculation. It is known that testosterone and hence possibly other endocrines of the gonadal system, congest the sebaceous gland and influence the behavior of the hair follicle.<sup>33</sup> Menstruation may be viewed, endocrinologically perhaps, as a repeated insult, against which the body immunizes itself. At least it may be associated with menotoxins or menotoxic manifestations of which Urbach<sup>33b</sup> has provided the most recent dermatologic, and Macht and his co-workers<sup>33</sup> the most massive general summary. Flares of infective processes on the skin (acne, staphylodermas) are known to occur in the premenstrual hydration period associated with the circulation of progesterone in the blood.<sup>32</sup> The relation of hydration to skin infection, especially pyogenic, has been discussed by the Reviewers elsewhere.<sup>32</sup> While it is not proposed to carry over what is known for the skin to other body tissues or infection in other structures, or to the allergic state at large, it is conceivable that recurring hydration and menotoxism so to speak, can underlie the gradual development of the infection hypersusceptibility and excessive reactivity of the victim of lupus erythematosus. Further reports on so radical a procedure as castration (induced menopause) in women in the child-bearing years will probably accumulate slowly but will be watched with interest.

\* Data presented before national meeting of American Coll. Phys., Nov. 19, 1943, to be published.

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PROTEIN-BOUND IODINE IN MICROGRAMS PER 100 Gc.	
Animal dialyzer	Plasma
Dog A,	4.5
	5.6
	5.0
	5.5
	5.2
	4.9
	6.4
	7.4
	11.2
	7.2
Average	6.34
Dog B,	5.4
Man A,	4.5
Man B,	4.2
Cow,	5.0
Horse,	6.8
Rabbit,	9.2
Cat serum,	11.1
	7.6
	6.34
	6.19

PROTEIN-BOUND IODINE IN MICROGRAMS PER 100 Gc.

Protein-bound Iodine in Erythrocytes and Plasma and Elsewhere. By J. F. McCLENDON and Wm. C. FOSTER (Department of Physiology, Hahnemann Medical College). In 1938 we observed that precipitation of blood proteins with cold methanol and washing the precipitate with cold acetone completely removed added inorganic iodide but retained all the iodine of added thyroglobulin. In 1941 we showed that tissues powdered in liquid nitrogen gave similar values of protein-bound iodine. How could the thyroglobulin penetrate cells? We dissolved low iodine thyroglobulin from a goiter in bicarbonate-Ringer and stirred it with iodine crystals at 38° for 20 hours. The thyroxine and total iodine content had greatly increased. Perhaps proteins are iodinated inside tissue cells. (The effect of this thyroglobulin on the basal metabolic rate of rats had also increased.) Our method gives similar results to that of dialysis or precipitation of protein with zinc hydroxide. The following table indicates that the determination of protein-bound iodine in plasma has no advantages over its determination in whole blood as the corpuscles contain about as high a concentration as the plasma.

PHYSIOLOGY  
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SESSION OF FEBRUARY 15, 1944

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**Electrical Potential of Human Skin Measured on the Fingers.** By T. CURRIE BARNES (Department of Physiology, Hahnemann Medical College). Finger-tips were placed in cups of saline connected by salt bridges to silver chloride electrodes leading to a Micromax recording potentiometer. Fifty right-handed males had an average potential of 0.84 mv. positive on the right fingers measured against the homologous fingers of the left hand; 37 of these subjects were predominantly positive on the right side. Twenty left-handed males had an average finger potential of 0.13 mv. positive on the right; 6 of these subjects were predominantly positive on the left and 5 were isoelectric. These results support the data of Snodgrass (50 right-handed females with average of 1.7 positive on right and 4 left-handed females positive on the left). Warming the skin made the potential more positive, augmented when the circulation was arrested by a cuff. At room temperature blocking the circulation usually produced negativity (the skin temperature fell to 28° C. or lower).

Muscular contraction (with or without circulation) made the finger potential positive (5 to 15 mv.) due to pressure on the cup serving as electrode. Clamping the relaxed finger gave the same effect. Pressure makes contact with the positive inner surface of the skin (Barnes, *Proc. Fed. Am. Soc. Exptl. Biol.*, 1, 6, 1942). Ethyl chloride, Sanborn electrode paste, camphor, chloroform and aluminum chloride made the skin negative (2 to 18 mv.). Mustard plaster raised the skin temperature 2° C. and produced 5 mv. positive. Confirming Burt and also Melchior (*Zentralbl. f. Chir.*, 45, 598, 1918), skin wounds were positive (20 to 40 mv.). This positivity arises from the inner surface of the uninjured skin at the reference electrode (demonstrated by the change of potential produced by warming the skin). In the frog cutting off the foot made the stump 20 mv. positive to the intact opposite leg. In man, allantoin, urea and sulfanilamide powder delayed healing (which was accelerated by yeast extract).

**Alloxan Diabetes.** By W. B. KENNEDY and F. D. W. LUKENS (Cox Institute, Univ. of Penna.). Diabetes was produced by the intravenous administration of alloxan in doses of 200 mg. per kg. of body weight, according to the method of Dunn, Sheehan and McLeitchie (*Lancet*, 1, 384, 1943). Ten rabbits which survived exhibited diabetes of moderate severity, with blood sugars which ranged from 300 to 700 mg. per 100 ml. Urinary excretion of glucose varied between 15 and 70% of the calculated available glucose in the diet. Nitrogen excretion in the fasting diabetic rabbit was not significantly increased over that in the normal rabbit. The islands of Langerhans showed early widespread necrosis which progressed to atrophy. Renal lesions, observed by others, were not found although in 2 rabbits an elevation of the blood urea nitrogen was found at death.

**Excretion of Certain Newer Sulfonamides in the Bile and Urine.** By HARRY SHAY, S. A. KOVACHOV, H. SIEBERT and S. S. FELS (Medical Research Laboratory, Samuel S. Fels Fund, Philadelphia). This study deals with the elimination of a few of the recently developed sulfonamides—especially phthalyl-sulfathiazole—with the bile and their fate in the gall bladder.

In addition, sulfathiazole, succinylsulfathiazole (sulfasuxidine), and sulfanilylguanidine (sulfaguanidine) were also studied.

Thirty acute experiments were carried out in fasting dogs under sodium pentobarbital anesthesia. Gall bladder and common bile duct were cannulated; a ligature was so placed on the common duct as to permit an independent flow of bile from a part of the liver to the gall bladder and from another part to the common duct distal to the point of ligation. The drugs were instilled in suspension in saline through the duodenal cannula in a dosage of 0.2 gm./k. Hepatic bile and urine were collected continuously and fractionated into 2-hour samples, while peripheral blood was taken at the end of each 2-hour period and the gall bladder was emptied at the same time. Such periods of observation lasted for 8 to 10 hours after the duodenal instillation of the drug.

Since the available preparations of sulfasuxidine and sulfathalidine both contained small amounts of free sulfathiazole as contaminants, we had both the free and conjugated forms of the drug to deal with when these compounds were studied. Marshall and his associates and Carryer and Ivy have shown that sulfanilamide is not acetylated by the dog's liver; we found the same to be true for sulfathiazole and sulfaguanidine. All results after the 2-hour sample were averaged and averages are used in this report. The blood levels reached for sulfasuxidine and sulfathalidine were very low compared with sulfathiazole and even when compared with sulfaguanidine.

Average blood level (mg. per 100 cc.)	
Sulfathiazole	3.25
Sulfasuxidine	0.34
Sulfathalidine	0.19
Sulfaguanidine	1.12

Free sulfathiazole was concentrated in hepatic bile 2.3 times that of the blood level. While the "free form"—presumably also sulfathiazole—was concentrated in the bile after sulfasuxidine 2.4 times, after sulfathalidine, it was concentrated 11.4 times. These results appeared to indicate that sulfasuxidine was not broken down by the liver cell or bile while sulfathalidine was broken down to some extent. The liver appeared to excrete sulfaguanidine as a filtrate from the blood. The average blood and hepatic bile levels were identical at 1.12 mg. per 100 cc. Sulfasuxidine concentration (5.29 mg. per 100 cc.) in hepatic bile was of somewhat lower magnitude than that found for sulfathiazole (7.48 mg. per 100 cc.). The bile/blood ratios, however, because of the much lower blood concentrations of sulfasuxidine were considerably greater for sulfasuxidine (38.3) than for sulfathiazole (2.3). In the case of sulfathalidine this difference was even more exaggerated. Average sulfathalidine in hepatic bile was 38.8 mg. per 100 cc. and the average bile/blood ratio was 206. The introduction of phthalic acid into the molecule greatly increased the elimination of the drug by the liver over that resulting from the introduction of succinic acid.

The gall bladder with all the drugs used except sulfaguanidine concentrated the drug brought to it with the hepatic bile in proportion to the water absorbed. This was established by finding that bile pigment and the drugs were concentrated in parallel by the gall bladder. There was no evidence of either absorption or excretion of the drugs by the gall bladder. With sulfaguanidine, there appeared to be some absorption of

the drug by the gall bladder since the gall bladder bile/hepatic bile ratio for the drug in 2 dogs were 2.5 and 1.5 as compared with their respective ratios for bile pigment of 4.3 and 3.1.

In the urine, sulfathiazole and sulfaguanidine appeared to be excreted by the glomerular filtration mechanism. There is accumulating evidence that sulfathiazole is handled by the kidney in such a manner. However, the concentration ratios (U/P) for sulfasuxidine and sulfathalidine reached such high levels that we suspect that these compounds are excreted by the tubules as well as by the glomeruli. However, a final conclusion must await the results of simultaneous creatinine and drug clearance studies which are now under way in our laboratory.



# BOOK REVIEWS AND NOTICES

**MEDICAL RADIOGRAPHIC TECHNIC.** Edited by GLENN W. FILES, Director of General Electric X-Ray Corporation Technical Service Department. Pp. 365, 381 figs. Springfield: Charles C Thomas, 1943. Price, \$6.00.

The contents of this book are largely divided into 3 categories: (1) Fundamental electrical concepts concerning the various units of the Roentgen ray equipment; (2) Processing; (3) Radiographic technique, with an excellent chapter on osteology as it concerns the radiographic technician. The positions illustrated are the routine positions utilized largely by the Technical Service Department of the General Electric Corporation. The portraits are magnificent and they have utilized diagrams and photography and Roentgen illustrations to good advantage.

This book on medical technique is in no way complete, however, and it is most unfortunate that Mr. Files has not utilized many methods that are conventional in general Roentgen ray departments. For instance, it has been recognized for years that the examination of the paranasal sinuses and of the head should be made in the erect posture. This, of course, means that if the book is widely used, many radiographic technicians will automatically plan their examinations in the horizontal posture, with the result that diagnoses, readily made from films of the erect posture, will become more difficult or be missed entirely.

The Reviewer does not mean to condemn this book by the above comments; he would recommend that it be in the hands of every radiographic technician and in the radiographic rooms of all Roentgen ray departments. It is to be hoped, however, that in the second edition of this book, that the authors will seek the help of some of their medical friends concerning additional radiographic technique that might, with propriety and extreme usefulness, be included in such a volume. The publisher has maintained his usual standard in this book. The figures and roentgenograms are magnificent. The paper is excellent and the print is easy to read.

**A HUNDRED YEARS OF MEDICINE.** By C. D. HAAGENSEN and WYNDHAM E. B. LLOYD. Pp. 444. New York: Sheridan House, 1943. Price, \$3.75.

WYNDHAM LLOYD's "A Hundred Years of Medicine" was published as a book of 344 pages in 1936. Now, C. D. HAAGENSEN, a surgeon-pathologist at Columbia who has long been concerned with medical history—has substantially revised all but the first fourth of the work and added chapters on the more recent paths of progress, such as chemotherapy, vitamins, thoracic surgery, and a new Part IV on New Social Aspects of Medicine. In Part I, "Medicine up to a Hundred Years Ago" (55 pages), brief high lights are given chiefly of the 18th century, on such topics as origins and theories of disease, scientific progress, surgery, hospitals, infectious diseases, the medical profession.

Medical Science of the past century (Part II) and Surgery for the same period (Part III) make up the bulk of the book. Even educated physicians will be surprised to realize how much of the story of medicine falls into this period—the new pathology, the germ theory, aids to diagnosis, chemotherapy, tuberculosis, vitamins, pernicious anemia, diabetes, cardiovascular disease,

nephritis. Such are the subjects picked to illustrate the century of medical progress, with an equivalent list for surgery. Virchow, Pasteur, Koch, Rollin, Ehrlich, Domagk, Laue (incidentally he no longer falls within the century limit), Trudeau, Banting, MacKenzie are the medical leaders portrayed both in illustration and text, with McDowell, Morton, Lister, Halsted, Semmichweis, Hugh Thomas, Harvey Cushing, in Surgery.

In the new Part IV, *New Social Aspects of Medicine*, Dr. Haagensen, after a brief summary of the progress made in advancing public health, departs from the role of historian to give an interesting, simple yet adequate presentation of the doctor's dilemma—how best to use our great mass of medical knowledge for the benefit of society, in order to bring the best, even though expensive, medical care within the grasp of all the people. Government support of hospitals, sickness insurance (governmental and voluntary), group practice, the Baker Memorial, are presented in a non-committal way that is especially timely now when the Wagner-Murray Bill threatens this country.

Thus, in phraseology too simple to repel even the simplest reader, is presented the progress of medicine during a period when it has advanced most rapidly in all its history—a leading science in an age of science and the one that affects individuals most acutely. The story is accurately told, with perhaps too much emphasis here and there on the author's favorites. The text is entertainingly presented, more so than in the earlier edition, and spiced with a good proportion of personal details. A defect easily remedied in later editions is that the illustrations appear entirely outside of the text that they are intended to illustrate: thus, Laue's portrait faces the discovery of insulin; Banting's portrait and *The Coming of Anesthesia* make strange bedfellows; while the Curies keep surprising company with problems of specialization in medical practice. However, this is indeed a minor defect in an otherwise excellent book.

E. K.

*Medical Clinics of North America*. Philadelphia Number (November 1943). Symposium on Medical Emergencies on the Home Front. Pp. 280. Philadelphia: W. B. Saunders Company, 1943. Price, \$16.00 per year. This number of the *Medical Clinics* maintains the proven worth of this series of monographs. The busy war-time practicing physician will appreciate this volume for it makes immediately available practical information which otherwise must be culled from a variety of sources at a cost of considerable time. One might not agree with individual points of view concerning certain specific therapies, but in general each author has done a commendable and practical job. Among the original contributions offered in this issue, the article on Sulfamazine by Dr. Flippin and associates is worthy of note, for it seems evident that this new sulfonamide drug will occupy an important place in prophylaxis and therapy.

*ELECTRONIC INTERPRETATIONS OF ORGANIC CHEMISTRY*. By A. EDWARD REICH, Ph.D., Assistant Professor of Chemistry, Wayne University, Detroit. Pp. 474. New York: John Wiley & Sons, Inc., 1943. Price, \$4.50.

This book will contribute to the annals of those organic chemists who, for obvious reasons, are dependent upon empirical knowledge and intuitive reasoning for the successful prosecution of their research. Those who are unfamiliar with modern electronic theory and the numerous applications of modern physical chemistry to organic chemistry will find this book invaluable as an introduction to the field. To avoid misunderstanding, the Reviewer hastens to add that the subject matter is extensively reviewed and discussed in a critical manner.

The first 3 chapters are devoted to a historical consideration of valence and

chemical affinity, ending with applications of the Lewis theory to problems of molecular structure. The development by the English school and the foundation upon which it rests are reviewed critically and presented in logical fashion in the following 2 chapters. There is an extensive discussion of the many applications of these theories to organic reactions and behavior. The remaining chapters are devoted to a critical discussion of the manner in which modern physics and physical chemistry have supported and extended these applications. An imposing number of organic reactions are critically discussed from this point of view. The appendix contains elementary presentations of the Lewis theory, reactivity, dipole moments, reactions in non-aqueous solutions, and modern theories of acid-base relationships.

The author has attempted to organize those concepts which are sound into a set of working rules for the organic chemist; he speaks the language of the organic chemist as much as it is possible for one to do so. Although there is undoubtedly much debatable ground and the ever-present danger of oversimplification, nevertheless the end justifies the means. To the advanced student as well as to many organic chemists, this book will open new horizons.

S. G.

THE BRITISH ENCYCLOPEDIA OF MEDICAL PRACTICE, including Medicine, Surgery, Obstetrics, Gynecology, and Other Special Subjects. MEDICAL PROGRESS, 1943 (Pp. 382); CUMULATIVE SUPPLEMENT, 1943 (Pp. 342; 5 figs.). Under the General Editorship of Sir HUMPHRY ROLLESTON, BART, G.C.V.O., K.C.B., M.D., D.Sc., D.C.L., LL.D., Emeritus Regius Professor of Physic, Cambridge; Sometime President of the Royal College of Physicians of London. London, Eng., and Toronto, Can.: Butterworth & Co. (Publishers), Ltd., 1943.

According to their customary procedure, the publishers have issued their 1943 cloth-bound volume of Medical Progress, together with the Cumulative Supplement, which is bound in stout cardboard.

In the Medical Progress volume—the 4th annual supplement—Part 1 presents 16 signed critical surveys of timely subjects; Part 2, a single article on Recent Developments in Drug Therapy; and Part 3—the larger part of the book—a few hundred brief summaries, arranged alphabetically. For instance, Herpes (based on 2 articles), Histoplasmosis (based on 2 articles) and Hodg-kin's Disease (based on 7 articles) together require  $2\frac{1}{4}$  pages.

The Cumulative Supplement aims to contain "fresh knowledge of a type likely to remain established." Its system of Key Numbers, at the top right-hand corner of each page, facilitates use in connection with the original volumes, which are key-numbered in the same way. As to the examples selected above, Herpes and Histoplasmosis are covered by 2 lines each of cross-references, whereas Hodgkin's Disease receives 3 pages.

Though such books are practically impossible to evaluate and report upon in writing, it appears to the reviewer that literary British medicine continues to "pull its weight" remarkably well in spite of the grievous handicaps of war conditions.

E. K.

UROLOGY FOR NURSES. By OSWALD SWINNEY LOWSLEY, M.D., F.A.C.S., Director of the Department of Urology (James Buchanan Brady Foundation) of the New York Hospital, and THOMAS JOSEPH KIRWIN, M.D., F.A.C.S., Attending Urologist of the Department of Urology (James Buchanan Brady Foundation) of the New York Hospital. New Printing. Pp. 493; 108 illus. Philadelphia: J. B. Lippincott Company, 1943. Price, \$3.00.

The authors present a new printing of their already popular text on urologic nursing. Much new material, covering recent developments in urology, is given. Throughout the book the role of the nurse, as applied to the field of urology, is stressed. The precision necessary for good results in urology

requires that nurses taking care of such patients have special training in that field. A short introductory chapter gives the historical background of the development of urology as a special field in medicine. This is followed by a chapter on the psychic and social aspects peculiar to the field of urology. Laboratory tests and technical procedures used in diagnosis are presented in such a manner that the rôle of the nurse is emphasized. The many instruments used in urologic study are described and their care discussed. Beginning with the sixth chapter, the organs of the urinary and genital tracts are considered in their anatomic sequence from within outward, beginning with the kidneys and concluding with the external genitalia. The embryologic, anatomic and physiologic aspects of each organ are considered. These discussions are followed by information on the diseases of each organ. Again the nurse's part in the care of such diseases is stressed. A summary of recent urologic developments appearing since the first printing of this book in 1936 is given. Such information includes points concerning diagnosis, treatment, diets and technical procedures. It is fortunate that such a reference and text-book exists. The urologist knows so well the importance of an intelligent and well-informed nurse in the care of patients with urologic disease. Nurses, medical students and physicians interested in the care of urologic patients would do well to read this excellent book.

L. L. LAY.

**THE ARTHROPATHIES.** A Handbook of Roentgen Diagnosis. By ALFRED A. DE LORMIER, A.B., M.D., Colonel, Medical Corps, U. S. Army; Commandant, The Army School of Roentgenology, Memphis, Tenn. Formerly Director, Department of Roentgenology, Army Medical School, Washington, D. C. Pp. 319, 678 figs. Chicago: The Year Book Publishers, Inc., 1943. Price, \$5.50.

This book, the first of a series, contains a wealth of material beyond what might be assumed from its title. By convention, the term "arthropathy" has not been generally applied to conditions like congenital rickets, scurvy, fractures and neoplasms. It is to be hoped, therefore, that the unusual though logical choice of title will not limit its sale or usefulness.

The author, a man of tremendous experience, has succeeded in presenting a wealth of material briefly and satisfactorily; but the usefulness of this book will be greatly limited by what the Reviewer believes is poor publishing. The paper is so poor that unless the light strikes the print just right, one cannot see it well. The illustrations, although the originals may have been good, lose a tremendous amount of their value because of the nature of the paper used for portraying the illustrations. The illustrations are well set on the page, but their descriptions are marred by the curved arrows, the difficulty in finding the description of a given illustration, and the very small print used in the legend. One gets almost "woozy" in trying to connect the legend with the proper illustration; and when it is found one discovers the value of the illustration has been ruined by multiple curved arrows.

The author has in a masterly fashion developed the importance of regarding the Roentgen examination as a consultation; and he has indicated in detail the various roentgenologic factors that should be considered in analyzing the joints and the items of clinical importance with which the Roentgen findings should be correlated.

The Reviewer believes that this handbook is one of the best that has been prepared on this particular subject, and it is most unfortunate that the publisher did not use better paper and better print. It is to be hoped that in the succeeding handbooks this phase will be corrected. Page 129 is a good example of a place where larger print could have been used with ease without sacrificing anything. Likewise, on pages 72, 73, 77 and 116 one can see where too many curved arrows have been utilized.

In spite of the above criticism, I believe that this book is eminently worthwhile and should prove of value to anyone interested in this subject.

E. P.

A CATALOGUE OF THE MEDIEVAL AND RENAISSANCE MANUSCRIPTS AND INCUNABULA IN THE BOSTON MEDICAL LIBRARY. Compiled by JAMES F. BALLARD, Director, Boston Medical Library. Pp. 246; 23 figs. Boston: Privately Printed.

In the number and importance of its Medical Manuscripts and Incunabula, the Boston Medical Library has been *facile princeps* in this country since the acquisition of the Ballard Collection (179 incunabula) in 1931. With 52 ancient manuscripts (written before 1501?) and 674 Incunabula, well distributed over the field of 15th century medical literature, this collection still stands well ahead of those in the Surgeon-General's Library, The College of Physicians of Philadelphia and the New York Academy of Medicine. In fact, when one considers that in Klebs' *Incunabula Scientifica et Medica*, of the 3000 editions given, only 850 are listed as medical, and, further, that many of the rarer items in public institutions are permanently off the market, one realizes what a rich treasure awaits students in the field in Boston. Future acquisitions should be few and far between. All this, coupled with Mr. Ballard's, the Compiler's, recent completion of 50 years of service to The Boston Medical Library, constitutes more than ample *raison d'être* for the publication of this handsome "Catalogue."

The collection apparently will continue to follow Dr. Ballard's aim of reproducing, as far as possible, a learned 15th century physician's library. This, incidentally, gives the Introducer, Dr. Henry Viets, an opportunity to consider, in a few pleasant pages, "our knowledge of what such a hypothetical library might contain." One wishes that this section might have been further expanded.

The book is welcome, not only for its content, but for its highly creditable editing and publishing. The Preface and Introduction are informative, clear and entertaining. The method of presentation is good. A number for each item, both in the lists and the textual references, is very convenient, as are the concordances and the name index. The occasional annotations are helpful and the illustrations adequate. The Compiler's competence in this field is a far better guarantee of the scholarly accuracy of the text than any recommendation that the Reviewer could make.

E. K.

## NEW BOOKS

- Textbook of Anatomy and Physiology.* By CATHERINE PARKER ANTHONY, B.A., R.N., Instructor of Anatomy and Physiology, Lutheran Hospital, Cleveland, Ohio; Formerly Instructor of Anatomy and Physiology, Frances Payne Bolton School of Nursing, Western Reserve Univ. Pp. 400; 33 tables; 153 illus. (8 color plates). St. Louis: C. V. Mosby, 1944. Price, \$3.50.
- Oral Histology and Embryology.* Edited by BALINT ORBAN, Foundation for Dental Research of the Chicago Coll. of Dental Surgery, School of Dentistry, Loyola Univ. Pp. 342; 9 tables; 262 illus. (4 color plates). St. Louis: C. V. Mosby, 1944. Price, \$6.50.
- Strophanturin.* Clinical and Experimental Experiences of the Past 25 Years. By BRUNO KIRSCH, M.D., Formerly: Professor on the Medical Faculty of Cologne Univ. (Germany), Visiting Professor to the International Univ. in Santander (Spain), Research Fellow at Yale Univ. Pp. 158; 24 figs; 11 tables. New York: Brooklyn Medical Press, 1944. Price, \$4.00.
- What is Hypnosis.* Studies in Conditioning. By ANDREW SALTER. Pp. 88. New York: Richard R. Smith, 1944. Price, \$2.00.
- Medical Physics.* Editor-in-Chief, OTTO GLASSER, Ph.D., Head, Department of Biophysics, Cleveland Clinic Foundation; Professor of Biophysics, Frank E. Bunts Educational Institute; Consulting Biophysicist, University Hospitals of Cleveland, Cleveland, Ohio. Pp. 1744; many figs and tables. Chicago: Year Book Publishers, 1944. Price, \$18.00.

- Civilization and Disease*. By HENRY E. SIEGERS, M.D., D.LITT., LL.D., William H. Welch Professor of the History of Medicine in the Johns Hopkins Univ. Pp. 255; 52 figs. Ithaca, N. Y.: Cornell Univ. Press, 1943. Price, \$3.75.
- Clinical Tropical Medicine*. By Twenty-seven Authors. Edited by Z. TAYLOR, B.Sc., M.D., F.R.C.P., Assistant Clinical Professor, New York Post-Graduate Med. School, Columbia Univ.; Physician-in-Charge, Parasitology Service, Department of Health, City of New York; Consultant in Tropical Medicine, Ellis Island Hospital, United States Public Health Service. Foreword by WILLIAM A. SAWYER, M.D., Director, International Health Division, Rockefeller Foundation. Pp. 957; 121 figs; 11 tables. New York, London: Paul B. Hoeber, 1944. Price, \$14.00.
- Physical Foundations of Radiology*. By OTTO GLASSER, Ph.D., Professor of Biophysics and Head of Department of Biophysics, Cleveland Clinic Foundation, Cleveland, Ohio; EDITH H. QUIMBY, Sc.D., Associate Professor of Radiology (Physics), Coll. of Physicians and Surgeons, Columbia Univ., New York; LAWRENCE S. TAYLOR, Ph.D., Chief of X-ray Section, National Bureau of Standards, Washington, D. C.; and J. L. WEATHERSWAX, M.A., Philadelphia General Hospital and Graduate School of Medicine, Univ. of Penn., Philadelphia. Pp. 426; 95 figs; 43 tables. New York and London: Paul B. Hoeber, 1944. Price, \$5.00.
- The 1943 Year Book of Pediatrics*. Edited by ISAAC A. ART, D.Sc., M.D., Professor of Pediatrics, Northwestern Univ. Med. School; Attending Physician, Passavant Hospital; Consulting Physician, Children's Memorial Hospital and St. Luke's Hospital, Chicago. With the Collaboration of ARTHUR F. ART, B.S., M.D., Associate Professor of Pediatrics, Northwestern Univ. Med. School; Associate Attending Pediatrician, Michael Reese Hospital; Attending Pediatrician, Chicago Maternity Center; Attending Physician, Spaulding School for Crippled Children and La Rabida Jackson Park Sanatorium, Chicago. Pp. 448; 76 figs. Chicago: Year Book Publishers, 1944. Price, \$3.00.
- Old Age in New York City*. By HELEN HARDY BRUNOT. Pp. 128; many tables. New York: Welfare Council of New York City, 1944. Price, \$1.50.
- Behind the Universe*. A Doctor's Religion. By LOUIS BERMAN, M.D. Pp. 303. New York and London: Harper & Bros., 1943. Price, \$2.75.
- Medical Care of the Discharged Hospital Patient*. By FRODE JENSEN, M.D., Instructor in Medicine, Syracuse University College of Medicine; H. G. WEISKOTTEN, M.D., Dean and Professor of Pathology, Syracuse University College of Medicine; and MARGARET A. THOMAS, M.A. (Oxon.). Pp. 94; 4 tables. New York: Commonwealth Fund, 1944. Price, \$1.00.
- Maurice Arthus' Philosophy of Scientific Investigation*. Preface to De l'Anaphylaxie a l'Immunité, Paris, 1921. Translated from the French, with an Introduction by HENRY E. SIEGERS. Foreword by WARFIELD T. LONGCOPE. (Reprinted from Bulletin of the History of Medicine, 14, No. 3, p. 366, October, 1943.) Pp. 26. Baltimore: Johns Hopkins Press, 1943. Price, \$ .75.
- Authority in Medicine: Old and New*. By MAJOR GREENWOOD, D.Sc., F.R.C.P., F.R.S., Professor of Epidemiology and Vital Statistics in the Univ. of London. The Linacre Lecture, May 6, 1943. Pp. 32. Cambridge: University Press; London: Bentley House; New York: Macmillan, 1943. Price, \$ .40.
- The March of Medicine*. New York Academy of Medicine Lectures to the Laity, 1943. Pp. 151. New York: Columbia Univ. Press, 1943. Price, \$2.00.
- The Hippocratic Oath*. Text, Translation and Interpretation by LUDWIG EBERSTEIN. Supplements to the Bull. of Hist. of Med., Editor: HENRY E. SIEGERS, No. 1. Pp. 64. Baltimore: Johns Hopkins Press. Price, \$1.25.

*Occupational Lead Exposure and Lead Poisoning.* Report of the Committee on Lead Poisoning of the Industrial Hygiene Section, A.P.H.A. Pp. 67. New York: American Public Health Assn. Price, \$ .75.

A useful guide to (1) practical and effective measures for the recognition and prevention of hazardous lead exposure in industry; (2) methods for the differential diagnosis and treatment of lead intoxication, and (3) the factual requirements for medical legal purposes. A 6-page classified bibliography will guide the reader to further study.

*Baby Doctor.* By ISAAC A. ABT, M.D. Pp. 308. New York and London: Whittelesy House, McGraw-Hill, 1944. Price, \$2.50.

*Medicine and the War.* Edited by WILLIAM H. TALAFERRA. Foreword by WILLIAM T. HUTCHINSON, Executive Secretary, Charles R. Walgreen Foundation for the Study of American Institutions. Pp. 193; several figs. and tables. Chicago: Univ. of Chicago Press. Price, \$2.00.

*The Religious and Philosophical Aspects of van Helmont's Science and Medicine.* By WALTER PAGEL. Supplements to the Bull. of Hist. of Med., Editor: Henry E. Sigerist, No. 2. Pp. 44. Baltimore: Johns Hopkins Press. Price, \$1.00.

*Evolution y Function Biologica de las Proteinas.* (Six lectures given in the amphitheater of the School on May 4, 7, 11, 13, 18 and 20, 1943.) By JUAN MENDOZA, Physician of the J. M. Ramos Mejia Hospital. Pp. 139; 4 charts. Buenos Aires: Guillermo Kraft, 1943.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES  
 MAY, 1944  
 ORIGINAL ARTICLES  
 CLINICAL ASPECTS OF PAIN IN THE CHEST  
 I. ANGINA PECTORIS  
 BY TINSLEY R. HARRISON  
 WINSTON-SALEM, N. C.  
 (From the Department of Internal Medicine of the Bowman Gray School of Medicine of Wake Forest College)

Thoracic distress is a common complaint. Thus, of the past 200 adult, non-obstetrical patients admitted to the North Carolina Baptist Hospital, 55 have complained of pain in the abdomen, 28 of pain in the chest, and 20 of pain in the head. When only patients admitted to the medical service are considered, the corresponding figures are 57, 50 and 38, respectively. It may be stated, therefore, that pain in the region of the chest is present in 15 to 25% of all adult patients who are sufficiently ill to need hospitalization, and that discomfort in this area is probably more common than in any other region of the body except the abdomen.

The majority of conditions which cause pain in the chest are either of serious nature or of trifling significance. Unfortunately, the differentiation between the two groups of disorders, though sometimes easy, is often difficult and occasionally well-nigh impossible. Hence, disastrous mistakes in treatment and embarrassing errors in prognosis are frequently made. In view of the puzzling manifestations and frequent occurrence of this symptom a study has been made of a large group of patients with chest pain.

The investigation, of which the present report is the first part, has lasted for a number of years and has dealt with several hundred patients. A complete history and physical examination have been made in all cases, and electrocardiograms and roentgenologic studies have been carried out in most of them. Additional investigations have been made when indicated. As the study has progressed it has become more and more apparent that the history is, in most patients, the single most important method of examination, and in many patients is more important than all other procedures combined. Therefore, ispecial emphasis has been placed on an attempt to analyze the subjective aspects of chest pain.

**Methods.** Most of the patients have been observed by the author personally, many of them on the wards of the North Carolina Baptist Hospital. Some have been seen in consultation, and a small number of the cases analyzed have been taken from records which were kindly loaned by professional colleagues. Since the purpose of the study has been primarily to try to assist in developing better criteria for separating the pain due to disturbance in the coronary circulation from that due to other disorders, patients with pain limited to the back of the chest and those with discomfort due to pleurisy brought about by acute infections have been omitted from the series. A few patients with pain limited to the shoulders or arms have been included, even when there was no pain in the chest.

The method of study has consisted of an unusually careful analysis of the pain, including detailed questioning concerning location, radiation, duration, intensity, quality, and above all, concerning the relationship of the pain to various body functions. A given patient's pain has been classified and put into a special category only when the causation of the pain has seemed to be determined with reasonable accuracy, as the result of all the studies made of the patient. In many instances it has not been possible to make a satisfactory diagnosis; some of the most baffling and interesting cases have remained puzzles and hence are not included in the series.

Angina pectoris has been selected as the subject of the first report, not only because this disorder embraced the largest single group of patients, but also for the purpose of obtaining a standard for comparison with other disorders causing pain which may to some extent simulate that due to coronary disease. Since the term "angina pectoris" is still used somewhat differently by various authors, it may be well to define at the beginning the meaning attached to it in this communication. By the term "angina pectoris," I refer to a condition characterized by recurrent attacks of discomfort in or near the chest, commonly induced by conditions which impose an additional burden on the heart, ordinarily dependent on disturbance of the oxidative processes in the myocardium and always attended by the likelihood of sudden death. Hence, pain due to myocardial infarction is excluded from consideration in this report. Certain patients with angina pectoris, as herein defined, have likewise been omitted. Thus, subjects with anginal attacks occurring as prodromal events prior to myocardial infarction, and patients with "status anginosus" are not included. These acute conditions, although falling within the category of angina pectoris, may be more properly studied in relation to myocardial infarction, with which they are closely allied, rather than in connection with the ordinary form of angina pectoris, which is a much more chronic disorder.

Since, according to the definition which has been given, the anginal syndrome is delimited in terms of clinical and functional rather than structural criteria, a critical reader will feel justified in entertaining some question as to the accuracy of the diagnosis. Granting the likelihood that a few of the patients included in the series may have been erroneously considered to have been suffering from angina pectoris, it seems improbable that the percentage of diagnostic error could be large. The reasons for this statement are as follows:

1. Of the 77 patients with whom this reports deals, 34 presented the picture of myocardial infarction either prior to or—more commonly—subsequent to the development of angina pectoris.

2. The manner of death of the 13 patients in the series who are known to be dead was such as to support the diagnosis of angina pectoris. Thus:

- (a) Of 8 patients who died outside the hospital
  - (1) Four died suddenly while in their usual state of health.
  - (2) Two died during typical status anginosus.
  - (3) One died suddenly following myocardial infarction.
  - (4) One committed suicide several weeks after myocardial infarction.
- (b) Of 5 patients who died in the hospital:
  - (1) One died as the result of embolism following coronary thrombosis, no autopsy being done.
  - (2) Autopsy revealed extensive disease of the coronary arteries in the remaining subjects.

Since the criteria employed for the diagnosis of angina pectoris were the same in the remaining subjects as in these 13 individuals, it is perhaps safe to assume that complete data would eventually reveal a similarly high incidence of sudden death and of coronary disease.

3. During the period in which these 77 patients were being studied there were: (a) Only 3 cases diagnosed as angina pectoris in which the subsequent clinical course was such as to cause this diagnosis to be changed. (b) Only one patient in whom at autopsy the diagnosis was not supported. (This patient had rupture of an aortic aneurysm but no significant coronary disease and no lesion of the aortic valves.) These 4 cases are not included in the series.

These observations lead to the conclusion that the percentage of erroneous diagnoses in the series is small, probably smaller than in the case of most conditions in which the diagnosis is based on clinical findings alone.

**Etiologic Factors.** The data listed in Table 1 are not materially different from those found in the publications of others, in that the predominance of males and of patients over the age of 50—both points stressed by Heberden in his original description<sup>16</sup>—are well illustrated. The generally accepted outstanding significance of disease of the coronary arteries is illustrated in the Table, as is the importance of diseases of the aortic valve, which was stressed by Keefe and Resnik.<sup>18</sup> The 3 patients who had no evidence of structural cardiac disease are of particular interest and will be discussed in some detail later. Since the etiologic factors were, in general, those which are generally recognized in the literature, they need not be considered further.

**Analysis of the Pain.** The data presented in Table 2 are largely self-explanatory. Localization of the distress in the substernal region, which has been emphasized as one of the most common features of the disease,<sup>3</sup> was far from a constant feature, being present in only a little more than half the patients. Many of the subjects complained of pain situated chiefly in the precordial area just to the left of the sternum. Abdominal localization of the pain was less frequent than some authors have indicated, and a number of the patients who on first being questioned stated that the pain was in the abdomen, were found on more careful questioning to have pain in the retro-xiphoid region. Pain in the region of the umbilicus or below it was not encountered in any

subject and the 4 patients who had definite epigastric localization of the pain also had discomfort in the chest or arms. In not one patient, therefore, was the pain exclusively abdominal. This point may perhaps be of some value in differential diagnosis. Several of the patients with precordial pain had discomfort in the region of the apex but not a single individual complained of pain entirely limited to the peripical area. Discomfort in the mid- or posterior axillary region was not encountered and no patient had pain in the right side of the back of the chest or in the flank. The rarity of pain in the region of the left costal margin is noteworthy. Perhaps the most confusing patients were the 2 who had pain limited to the back of the neck. The other 4 patients with pain in the back of the neck also had pain in the chest or arms.

TABLE 1.—ETIOLOGIC DATA IN 77 PATIENTS WITH ANGINA PECTORIS

I. Sex		II. Age		III. Underlying Disease Process	
No. of cases	%				
75.3		20-29	3	Coronary arteriosclerosis (presumptive or proven)	64
24.7		30-39	5	Lesions of aortic valve:	
		40-49	9	Rheumatic	4
		50-59	26	Calcareous	2
		60-69	27	Syphilitic	1
		70-79	7	Syphilitic aortitis and (presumptive) aortic stenosis	1
				Thrombo-angitis obliterans	2
				No evidence of structural cardiac disease:	
				Hypoglycemia	2
				Hypochromic anemia	1

The lack of sharp localization which, as stressed by Lewis,<sup>20</sup> is a feature of pain arising from visceral structures, was noted in most of the patients. This point is of some value in differentiating the pain of angina pectoris from that of cardiac neurosis, as has been pointed out by Goodrich and Keyes.<sup>12</sup> Eleven subjects stated that the point of greatest intensity was extrathoracic, but in only 5 of these was there a complete absence of substernal or precordial discomfort. Such cases, with pain limited to extrathoracic areas, have been recently studied by Spillane and White,<sup>25</sup> who found that, after varying periods of time, pain in the chest developed in each of their 12 patients who originally had the pain of effort only in the arms. In recent years increasing evidence has accumulated indicating that the cardiac afferent pathways may be more numerous and widespread than was formerly believed, and suggesting that various accessory pathways may be responsible for the bizarre distribution of the pain often encountered in patients with angina pectoris.<sup>1,5,22</sup>

The chief points of interest in regard to the *duration* of the pain were the rarity of pain lasting for more than 30 minutes, and of discomfort enduring for only a few seconds. There were 3 patients who originally stated that their pain lasted for a fraction of a minute only, but when pain produced under observation by exercise was timed, it was found to last for a minute or longer in each instance. The 3 patients who complained of pain lasting for days at a time all admitted having paroxysmal exacerbations which were brought on by the usual precipitating factors and which lasted only a few minutes. Perhaps these patients had the so-called myocardial fatigue pain plus angina pectoris, but if so, they were unable to distinguish the two pains from each other in respect to location and quality.

In the case of the subject who claimed to have attacks lasting from 1 to 2 hours, the situation was complicated by the existence of a well-marked anxiety state as well as attacks of paroxysmal tachycardia, either of which may have caused precordial pain for a considerable period of time. However, she claimed that nitroglycerine relieved these pains as well as the more common and shorter anginal attacks produced by effort or emotion. The patient with attacks due to paroxysmal auricular fibrillation also had frequent attacks of shorter duration brought on by effort. Of the 3 persons with attacks due to hypoglycemia and lasting 30 minutes to 1 hour, one had frequent attacks of shorter duration appearing with effort. The other 2 had no attacks with effort and apparently in these instances hypoglycemia was the sole precipitating factor, the patients being free from any signs of structural cardiac disease. These subjects will be discussed in more detail later.

The tendency of anginal attacks to last for only a few minutes was mentioned by Heberden,<sup>16</sup> and its diagnostic significance has recently been reemphasized by Barnes and Pruett.<sup>3</sup> Subject to the error inherent in all generalizations, it may be stated that a pain lasting for only a few seconds or for more than an hour is not likely to be due to angina pectoris.

The data as regards *intensity* of pain are somewhat at variance with widely accepted concepts. Severe pain was rare in this series. In approximately half the patients pain was either mild or minimal (*i. e.*, barely perceptible). Most such patients did not seek medical advice because of pain in the chest but because of some other complaint such as hypertension, and in these subjects with mild pain the presence of angina pectoris was demonstrated only as a result of careful questioning. However, mildness of the pain does not in any sense speak against angina pectoris. One of the patients with pain of minimal severity expired suddenly when apparently in his usual health, and another who died some years later of cerebral hemorrhage was found at autopsy to have well marked coronary sclerosis. Unless the physician makes a deliberate attempt to inquire carefully about minor chest discomfort appearing with effort and lasting a few minutes in all patients beyond the age of 50—whether or not such a complaint is offered voluntarily—

he will overlook a large percentage of the individuals with angina pectoris.

TABLE 2.—ANALYSIS OF PAIN IN 77 PATIENTS WITH ANGINA PECTORIS

I. Location and, or, Radiation

No. of cases	%	
42	54.5	Substernal
30	39.0	Precordial
23	29.9	Left arm (including shoulder and hand)
14	18.2	Front of neck
12	15.6	Both arms
6	7.8	Retro-xiphoid
6	7.8	Back of neck
5	6.5	Back of left side of chest
4	5.2	Epigastrium
3	3.9	Left pectoral
2	2.6	Right arm (including shoulder and hand)
1	1.3	Jaw
1	1.3	Left anterior costal margin
		Note absence of pain limited to periaxillary region or axilla, or localized in right side of back or abdomen, lower abdomen, lower back or flanks.

II. Duration

No. of cases	%	
0	0	Less than 1 minute
69	89.6	1 to 30 minutes
4*	5.2	30 to 60 minutes
1†	1.3	1 to 2 hours
3‡	3.9	Days or weeks
		* Three cases had hypoglycemia—1 had paroxysmal auricular fibrillation.
		† Paroxysmal tachycardia and cardiac neurosis in addition to effort angina.
		‡ Dull, steady pain with exacerbations lasting a few minutes.

III. Intensity

No. of cases	%	
7	9.1	Minimal
31	40.2	Mild
22	28.6	Moderate
10	13.0	Severe
7	9.1	Variable

IV. Character

No. of cases	%	
31	40.2	Constrictive*
15	19.5	Aching
4	5.2	Burning
1	1.3	Numbness only
1†	1.3	Lancinating
0	0	Throbbing
25	32.5	Vague
		* Including "squeezing," "tight," "cramping," "heavy," "pressing," etc.
		† This patient had pain in the chest from gall-bladder disease as well as from angina pectoris.

The data as regards the *character* of the pain are not very satisfactory in that one-third of the patients were unable to describe this feature accurately. (In order to avoid obtaining a misleading description of the pain the patients were given a list of descriptive terms, such as "stabbing," "shooting," "burning," "squeezing," "grasping," "grip-ping," "aching," "throbbing," "pressing," "heavy," and so forth, and

allowed to choose the term which best described the discomfort.) As which fell under the general category of constrictive or "pressing" pain. However, about one-fifth of the patients described the discomfort as consisting of a dull ache, and several individuals insisted that the pain consisted of a feeling of warmth. This is of interest in view of the observations of Lewis,<sup>20</sup> which indicate that pain having a burning quality arose only in superficial tissues of the body and never in the internal organs. In 12 patients the sensation of pressure or aching in the chest was accompanied by numbness in other parts, particularly in the arms or fingers. One patient, whose discomfort was limited to the left arm, complained only of a sensation of numbness. From a diagnostic standpoint it is perhaps of interest that no patient described the pain as being synchronous with the heart beat, *i. e.*, of throbbing character, and that the only patient who claimed to have lancinating ("stabbing," "shooting," "cutting" or "sticking") pain also had gall-bladder disease producing pain in the left side of the chest. It is possible that this individual may have confused the pain from the two different disorders, but opportunity to question him when the pain was present did not occur. In addition there were 4 patients who complained of pain of stabbing quality, who are not included in the series because these individuals did not sufficiently fulfill the criteria for the diagnosis of angina pectoris. They are regarded as suspicious cases of the disorder and are being followed. Thus far, none of them has developed unequivocal evidence of the disease. Hence, these individuals are not included in the series. In any case, it may be stated that pain of lancinating character either does not occur at all, is due to associated disorders, or is at least very rare as a result of angina pectoris.

**Relation of the Pain to Various Body Functions. A. POSITION.** No patient noted that the standing or sitting postures tended to induce the pain. However, there were 11 individuals in whom the attacks of pain seemed to bear a definite relationship to the recumbent position (Table 3, part I). All of these subjects gave a story of pain on effort. In addition they had attacks of pain which came on frequently in the recumbent posture while at rest, but did not tend to have attacks coming on at rest while sitting upright or standing. Two of these 11 subjects also noted that when sitting in a slumped posture with the feet elevated the attacks tended to come on just as they did during recumbency. There were likewise 2 patients who had attacks of pain with effort due to angina pectoris, and attacks of pain when lying down, due to hiatal hernia. One of these patients was able to distinguish between the character and location of the pain. The other individual could make no such distinction, stating that the pain due to the two conditions was identical in distribution and in character. As an example of the rather striking relationship which exists in certain patients between anginal attacks and the recumbent posture, the following cases may be cited.

TABLE 3.—RELATION OF THE PAIN TO VARIOUS BODY FUNCTIONS IN 77 PATIENTS WITH ANGINA PECTORIS

I. Position as Factor in Inducing Attacks

No. of cases	%
Standing	0
Sitting (upright)	0
Sitting (slumped)	2
Reclumbent*	14.2
* One patient had aggravation by lying on left side with partial relief on turning to right side.	
† These patients experienced partial to complete relief by changing to standing or sitting position.	

II. Pain Induced by General Exertion

No. of cases	%
General exertion*	70
	90.8
* The patients with pain not related to effort included (1) 2 patients with hypoglycemia; (2) 2 patients with ectopic tachycardia; (3) 2 patients who led unusually sedentary lives; (4) 1 patient who was almost bedridden by thrombo-angitis obliterans.	

III. Pain Induced by Specific Local Muscular Movements

No. of cases	%
Specific local muscular movements	8*
	10.4
* These patients had "coronary shoulder pain" in addition to angina on effort.	

IV. The Alimentary Tract in Relation to Anginal Attacks

No. of cases	%
A. Functions tending to precipitate pain:	
Swallowing*	2
Eating:	
Alone†	6
Plus exertion†	17
Constipation	1‡
B. Functions tending to relieve pain:	
Eating	12
Vomiting	1¶
Belching	7
Expulsion of flatus	2
	2.6

\* The pain due to swallowing was caused by esophageal spasm in 1 patient and by cascade deformity of the stomach in the other.  
 † One of these patients did not have pain on effort—he took practically no exercise. The remainder had typical effort angina and also attacks at rest after meals.  
 ‡ Less exertion was required to induce attacks after meals than at other times.  
 § Attacks more readily provoked when constipated.  
 || Attacks most frequent 3 to 5 hours after meals, occurring with less effort—3 patients; or at rest—7 patients. Two additional patients without effort angina or evidence of structural cardiac disease had severe anginal attacks due to hypoglycemia.  
 ¶ This patient had chest pain due to (1) angina pectoris and (2) hiatal hernia. There were 4 patients—not listed in the table—with angina plus non-anginal chest pain induced by eating and relieved by belching and due to abdominal disorders.

V. Relation of Anginal Attacks to Certain Additional Factors

No. of cases	%
Attacks induced by emotion*	14
18.2	
Respiratory functions:	
Attacks induced by cold†	10
13.0	
Breathing	1‡
Coughing	1§
1.3	
* Emotion induced seizures in 6 of 8 patients with aortic lesions.	
† Cold sheets (1), cold drinks (2), cold wind plus mild exertion (7).	
‡ Rheumatic aortic stenosis—suspicious signs of adhesive pericarditis.	
§ Congestive failure with severe attacks of coughing—pain produced by prolonged seizure only, e. g., by the exertion of coughing—not the act of coughing.	



VI. Nocturnal Pain: Analysis of Precipitating Factors\*

No. of cases	%
11	14.3
2	2.6
2	2.6
1	1.3
1	1.3
1	1.3
1	1.3
3	3.9
21	27.3
Total	
* Two additional patients had nocturnal pain due to hiatal hernia.	
Recurrent posture	
Spontaneous hypoglycemia	
Paroxysms of ectopic tachycardia	
Nightmares	
Cold sheets	
Paroxysmal dyspnea	
Unexplained	

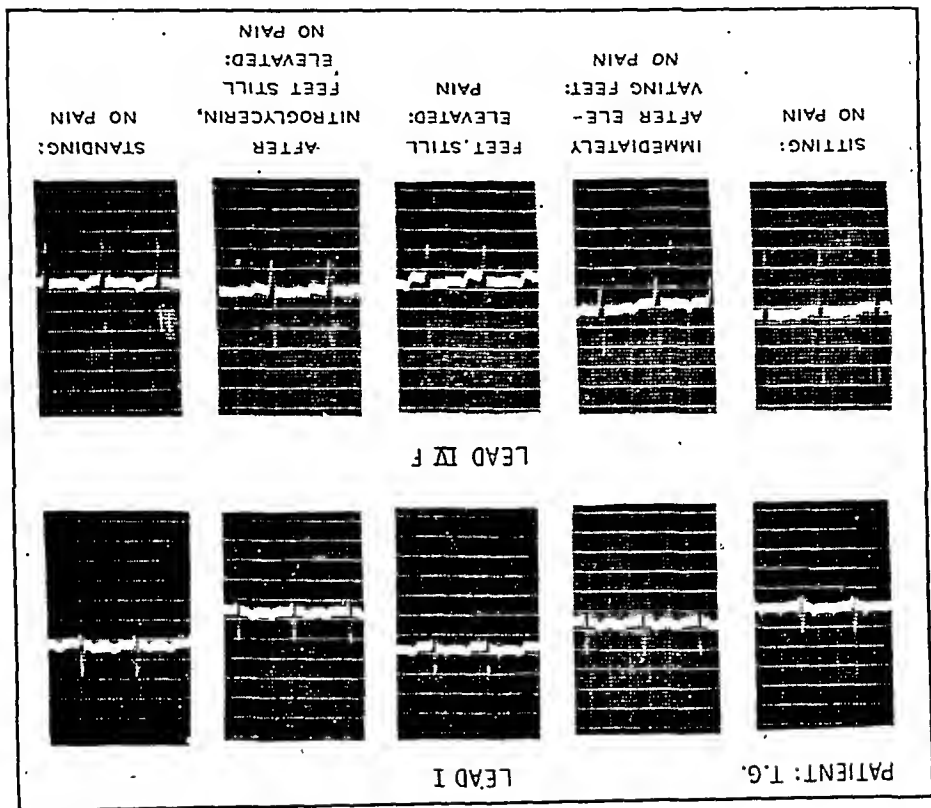


Fig. 1.—Patient T.G. The changes developing on elevation of the feet appeared slowly and hence were not the result of alterations in the electrical axis of the heart. Nitroglycerine relieved the pain and caused partial reversal of the electrocardiographic changes, which quickly returned to normal on assumption of the upright posture.

**Case Studies.** Case 1. A white male, aged 55, complained of a feeling of substernal pressure radiating to the left shoulder, brought on by walking and relieved within 3 to 8 minutes by rest. He had also noted that when he would lie down the discomfort would set in within a few minutes and would become progressively worse until he assumed the sitting posture, which was followed by gradual disappearance of the pain. He insisted that the discomfort produced by lying down was identical in location and character with that caused by effort. However, the discomfort in the recumbent position tended to last longer if he remained in this posture. Sitting in a chair with the feet elevated would likewise induce a typical attack, while sitting in a chair in an upright posture did not cause pain. Nitroglycerine relieved both the pain induced by

effort and that caused by recumbency. Repeated roentgenographic studies were made in various positions and also before and after exercise. It was found (Fig. 1) that the recumbent posture induced the same changes in the electrocardiogram which occurred on physical exertion. Even though he remained in the recumbent position the electrocardiographic changes tended to return to normal following nitroglycerine. Several months later, while walking slowly, the patient suddenly fell to the ground and expired.

This patient constituted a striking example of the *angina decubitus* of the older authors, that is, of anginal attacks occurring in the recumbent position. Even though this condition has been reported in the literature from time to time for many years, the frequency of the recumbent position as a precipitating factor does not seem to have been generally recognized. The point is perhaps of more than academic interest because it would seem to constitute evidence against the idea that the ideal treatment for angina pectoris is prolonged rest. My own experience in this matter is that the patients with angina pectoris are not only happier but actually seem to be healthier if they are allowed a reasonable amount of activity.

The mechanism of anginal attacks occurring in the recumbent posture is still unknown. Elsewhere,<sup>12</sup> I have suggested that the decline in blood pressure which tends to occur on assuming the recumbent position might, in a patient with rigid coronary arteries, induce myocardial anoxia, while the same decline in pressure would not, on the basis of theoretical hemodynamics, be expected to induce cardiac oxygen deficiency in a person with distensible vessels of the same caliber. In favor of such a mechanism is the case reported by Rubin.<sup>23</sup> His patient had an extremely labile blood pressure which would decline from 180/100 to 120/70 after a few minutes in the recumbent position. The patient did not have attacks during effort despite reasonable activity, which was limited somewhat because of the coexistence of intermittent claudication. He had frequent attacks which invariably came on in the recumbent posture. The author did not mention having taken electrocardiograms in different positions, but did cite records showing that the patient developed well-marked changes in the S-T segments and in the T waves when breathing a low-oxygen mixture. Some months later the patient expired suddenly.

A further investigation would seem to be needed of blood pressure changes and other alterations in circulatory dynamics in patients with angina of recumbency before any definite conclusions can be drawn as to the mechanism of such attacks. The following patient is an example of an individual who had pain during effort and also during recumbency, the attacks being dependent on two different disorders.

CASE 2. A white female, aged 65, had had several attacks of "pressing" pain localized just to the left of the sternum in the precordial area, while washing dishes, and two attacks while walking up-hill. Standing or sitting quietly brought relief in a few minutes. In addition she had had numerous attacks of similar pain coming on shortly after getting into bed. Five weeks before admission to the hospital she had a severe attack of pain lasting several hours, associated with an initial rise in systolic blood pressure to 180 mm. and followed by a fall to 120 mm., her usual pressure being approximately 160.

Several days later her physician heard a friction rub over the precordium which disappeared in 48 hours, and at that time the patient had slight fever. When she was seen some weeks later the physical examination was negative except for minimal hypertension and slight cardiac enlargement. Electrocardiogram revealed inversion of the T wave in the precordial lead. Fluoroscopic examination of the stomach showed a small hiatal hernia. The pain induced by recumbency was not relieved by nitroglycerine but was benefited by atropine.

It seems likely that in this patient the pain brought on by the recumbent position was due to the hiatal hernia even though it had the same character and distribution as the pain brought on by effort and due to angina pectoris.

Inquiries as to the relative effects of the various recumbent postures in producing attacks of pain were negative except in 1 patient who thought that the pain was worse lying on the left side and was partially relieved by turning on the right. Since this patient had, in addition to typical angina pectoris, a good many of the manifestations of anxiety neurosis, a condition in which precordial pain while lying on the left side is often encountered, the significance of the difference in the two positions is questionable. In any case it can be stated that the recumbent posture is a fairly frequent precipitating cause of anginal attacks, and also that a pain which tends to be aggravated by the sitting or standing position is in all probability not angina pectoris.

B. THE EFFECT OF MUSCULAR EFFORT (Table 3, parts II and III). Obviously a sharp distinction must be drawn between *general muscular exercise* such as walking, which puts no particular strain on any of the muscles in the region of the chest, and *specific muscular movements* such as bending the body, raising the arm, and so forth. It should be noted that slightly more than 90% of the patients in this group gave a definite and clear-cut history of pain induced by effort. Many of these patients had attacks which occurred under varying circumstances at rest. However, from a standpoint of diagnosis, the important feature was the story of the attacks which came with exertion. In most instances this history was readily obtained, but in a few cases it would be elicited only after very careful inquiry, for in these latter instances the patient sought medical advice for some unrelated disorder. In such patients much finesse is needed in taking a proper history in order to avoid "leading" the patient and in order to maintain in the patient's mind a clear distinction between shortness of breath and a mild pressure sensation or similar discomfort.

The patients who had pain which was not related to effort are of particular interest, for it is this group of individuals who are likely to constitute the most difficult diagnostic problems, and in whom errors are often made. These subjects will be discussed in more detail later. In his original description of angina pectoris, Heberden<sup>16</sup> stressed the relationship of the pain to effort and the frequency of sudden death. Many later authors, losing sight of these cardinal features of the disease, began to apply the term "angina pectoris" to a motley array of unrelated disorders having nothing in common except the presence of discomfort in the chest. The state of confusion so engendered persisted for more than a century and was not dissipated until

the now classical work of Keefe<sup>18</sup> and Resnik<sup>18</sup> appeared. These authors stressed Heberden's original criteria, reconciled the conflicting "aortic" and "coronary" theories and pointed out that angina pectoris was a clinical entity in the sense of the course of the disease, was a physiologic entity in the unity of the causative mechanism of the pain, but was not a pathologic entity in that the same functional disturbance—myocardial anoxia—might be induced by a variety of morbid states.

Eight individuals complained not only of pain on general exertion such as walking, but of discomfort induced by specific movements of the muscles of the shoulder or arm. This pain not only differed from that of angina in regard to the condition inducing it, but was of longer duration, different intensity, and often of different quality, being typically aching and occasionally throbbing in character. Discomfort of this type following myocardial infarction has been described by several authors,<sup>2,4,8,17</sup> and a few<sup>2,18</sup> have mentioned it as occurring in patients with angina pectoris who have not had myocardial infarction. Presumably, it represents the reflex effect on skeletal tissues of the cardiac disorder. This type of pain may be very confusing because there are certain patients in whom this symptom is prominent and dominates the picture, while the pain of angina may be decided in the background. Such a case has been reported elsewhere.<sup>14</sup> The pain may be identical in character with that produced by various disorders of the shoulder joint or other skeletal tissues in the absence of coronary disease. The decision as to whether or not angina pectoris is present cannot be made on the clinical features of this referred pain but must be based on the presence of the typical anginal story. Since the question of "coronary shoulder pain" will be discussed in considerable detail in a future publication dealing with chest pain arising in the skeletal tissues, the matter need not be considered further now.

C. ANGINAL ATTACKS IN RELATION TO VARIOUS ALIMENTARY TRACT FUNCTIONS. The data are summarized in Table 3, part IV. It may be noted that anginal attacks were not accompanied by discomfort on swallowing in any patient. Two subjects who had dysphagia had other disorders in addition to angina pectoris. There was likewise little relationship to expulsion of flatus or to constipation, although the act of defecation occasionally induced seizures in 3 patients. The rarity of vomiting in relation to anginal attacks is also noteworthy. There were 4 patients who had abdominal disorders which induced non-anginal pain in the chest, the pain being completely or partially relieved by belching. However, in addition to these there were 7 other patients in whom no evidence of any primary abdominal disorder could be found, but who insisted that attacks were more readily induced by effort when there was abdominal distention, who occasionally had attacks occurring at rest and induced by distention and who had obtained either partial or complete relief by belching. These findings are not surprising when one recalls the recent studies of Gilbert, LeRoy and Fenn,<sup>11</sup> who have shown by means of the *thermostromuhr* that constriction of the coronary arteries of the dog may be reflexly induced by distention of the stomach. The same authors<sup>10</sup> showed that

in each of 3 patients with angina pectoris the ingestion of food caused a decrease of 20 to 40% in the length of time required to produce anginal attacks by breathing 10% oxygen. That the effect of meals was exerted—at least partially—through a reflex mechanism, rather than solely through increased cardiac work, was suggested by the following points: (1) atropinization prevented the effect of meals; (2) some patients could relieve the attacks produced by distention either by belching or by loosening the belt. It therefore seems to be established that abdominal distention may, in certain patients, be an important trigger factor in precipitating attacks of angina pectoris.

Since the time of Heberden, it has been recognized that eating often induces anginal attacks. Much less emphasis has been placed on the fact that eating may likewise tend to prevent the seizures. In this series there were 6 patients who had anginal attacks occurring at rest shortly after meals. Five of these also had typical effort angina. The other patient gave no history of a relationship to exertion, but led an unusually sedentary life. Seventeen subjects had noted that considerably less exercise was required to induce the attacks shortly after meals than several hours later, but none of them had attacks after meals if they remained at rest. *Twelve patients had found that the ingestion of food tended to prevent the seizures.* Ten of these had the usual complaint of pain on effort and, of them, 3 had found that approximately 3 hours after meals less effort was required to induce pain than at other times. The remaining 7 patients in this group had, in addition to attacks during effort which were not related to meals, occasional seizures at rest, the resting attacks occurring almost entirely in the period of 2 to 5 hours following food.

There were 2 patients who had never had anginal attacks with effort, but who had numerous severe attacks occurring at rest, never within the first 2 hours after eating. One of these patients had been reported elsewhere<sup>4</sup> as an instance of angina pectoris without structural cardiac disease and dependent on glucose deficiency. The chief findings in regard to the other patient may be summarized as follows:

Case 3. A 39 year old business man had had numerous attacks of constrictive pain in the region of the cardiac apex for 5 months, accompanied by pain and numbness of the left arm. The intensity varied from mild to very severe; the total duration of the attacks was 1 to 2 hours, during which the pain would recur in short seizures of a few minutes with an interval of comfort lasting several minutes. He led an active life, but on no occasion had an attack been precipitated by unusual effort, by emotion or by eating. Most of the attacks had set in during the night or in the morning before breakfast. Although he had many seizures, he could not recall a single one which had begun within 2 hours after a meal. Following the severe seizures he had repeatedly been observed to have normal temperature and leukocyte counts. Electrocardiograms had been entirely normal when taken between attacks but records obtained during seizures had repeatedly shown changes considered typical of anterior infarction (Fig. 2). He had been studied in several hospitals and diagnoses of neurosis, coronary thrombosis, and of "coronary spasm" had been made. Physical examination was essentially negative. Electrocardiograms were quite normal, both before and after strenuous exercise which produced no pain. Glucose tolerance curves displayed a decline from 154 (mg. "glucose")

per 100 cc.) at  $\frac{1}{2}$  hour to 60 at 3 hours. Fasting blood sugars varied from 100 (on a balanced diet) to 64 (on a high-carbohydrate diet). When put on a high-carbohydrate diet he had frequent mild seizures which had been absent for several days on a high-protein, low-carbohydrate diet. The administration of insulin—25 units—induced a mild seizure of pain and moderate change in the T waves.

PATIENT: P.V. NO PAIN PAIN

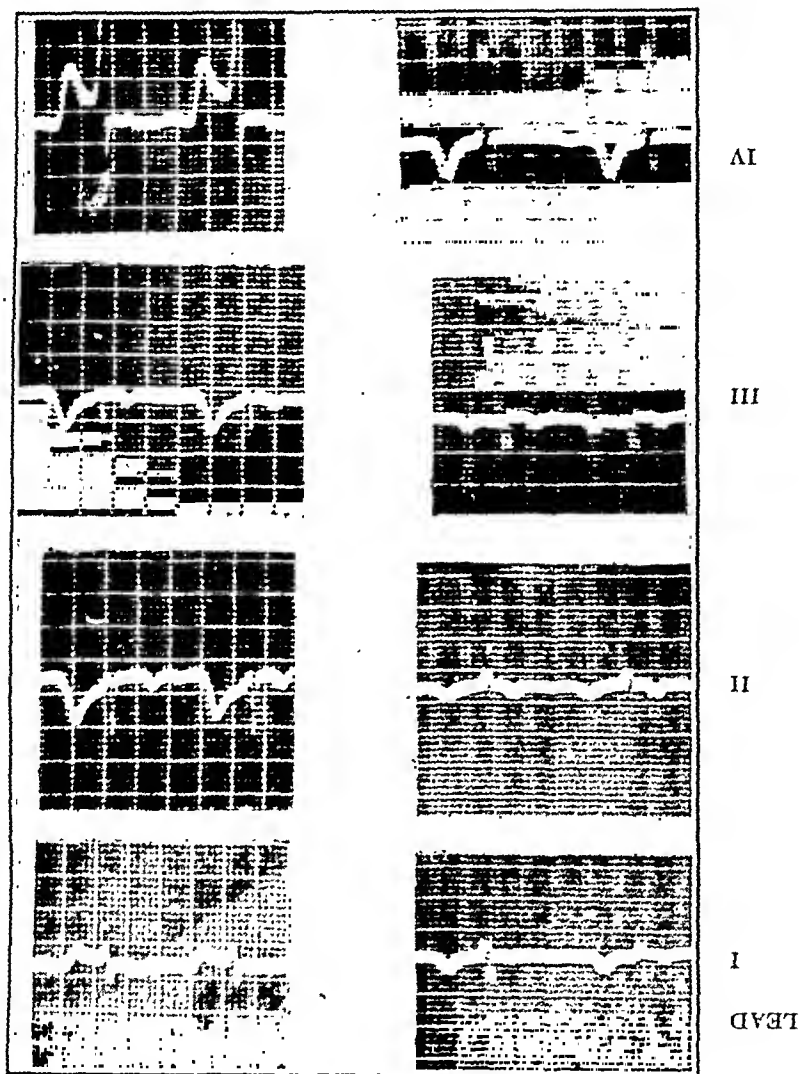


Fig. 2.—Patient P.V. The tracings on the left were taken during freedom from pain. Those on the right were obtained during an attack. The following day the record was again normal. Similar changes were repeatedly observed during an attack whether occurring spontaneously or induced by insulin.

*Comment.* In this patient the evidence seems reasonably complete that angina pectoris was due solely to hypoglycemia. The fact that strikingly low levels of blood sugar were not encountered does not speak against this assumption, for it has been shown<sup>15</sup> that symptoms due to glucose deficiency may occur in the presence of blood sugar levels within the lower limits of the normal range.

Since the relationship between glucose deficiency and anginal attacks has been discussed in some detail elsewhere<sup>5</sup> only a few points will be mentioned here. It has been known for more than a decade that overdosage of insulin may occasionally precipitate anginal attacks in diabetic patients with coronary sclerosis. Sippe and Bostock<sup>24</sup> were apparently the first writers to point out that spontaneous hypoglycemia may have a similar effect. Weinstein and Matlikow<sup>27</sup> recently reported 2 cases, one of whom had attacks during effort and emotion, but only provided these stimuli occurred shortly before a meal. During a glucose tolerance test this patient developed an anginal attack when the blood sugar level was 54 mg. per 100 cc., the pain being promptly relieved by glucose. The second patient was somewhat similar except attacks occurred frequently at rest as well as during exertion undertaken before meals. This individual had never had an attack except when hungry. He, likewise, developed an anginal attack during the glucose tolerance test, the blood sugar being 42 mg. per 100 cc. and relief occurring from glucose.

Patients such as Case 3, who have no evidence of structural cardiac disease\* but have anginal attacks precipitated only by glucose deficiency and never by effort, must be quite rare, as I have found no other instance in the literature except for the single case previously reported.<sup>15</sup> However, patients who have coronary sclerosis and in whom hypoglycemia is one of several precipitating factors, are common. In this series of patients glucose deficiency, while less important than physical effort and eating, was about equal in importance to emotion as a precipitating factor. The point is of some practical importance because ordinarily the tendency toward a low blood sugar level can be readily controlled by dietary measures.

D. EXERCISE AS A PRECIPITATING FACTOR. The data are summarized in Table 3, part V. Only a little more than 10% of the patients with angina due to coronary sclerosis had attacks occurring with emotion, in the absence of exertion. However, 6 of 8 patients with disease of the aortic valve had such seizures. The tendency of patients with "aortic angina" to have attacks independently of exertion has been stressed by Lewis,<sup>21</sup> and the mechanism may, as suggested elsewhere,<sup>18</sup> be related to a greater sensitivity of normal than of diseased coronary vessels to vasomotor influences.

E. COLD AS A PRECIPITATING FACTOR. Since most of the patients in this series lived in areas (North Carolina and Tennessee) with relatively mild climate, the influence of cold was, as might be expected, less striking than in the cases reported by most other authors. Even so, it was noted that an occasional patient had attacks from getting into bed between cold sheets, or from the ingestion of cold drinks, while a number of individuals found that walking against the cold wind induced the attacks more readily than otherwise.

\* Whether such individuals actually have minimal and "subclinical" coronary disease cannot be stated with certainty. The usual manifestations were lacking and one such patient—a young woman—has been asymptomatic for more than 6 years.

F. THE RELATION OF ANGINAL PAIN TO RESPIRATORY FUNCTIONS. The only patient who complained that the pain was aggravated by breathing was a young woman with aortic stenosis and signs suggestive of adhesive pericarditis. The single individual who had anginal attacks with relationship to coughing, had rather severe cough as the result of congestive heart failure, and at the end of a prolonged bout of coughing would have attacks brought on by the exertion involved. The negative relationship between anginal attacks and various respiratory functions is significant in differential diagnosis, for there are a good many patients who have—in association with pleural disorders—pain with location and radiation similar to that of angina. It can be stated as a general rule, to which there are possibly a few exceptions, that any pain which bears a direct relationship to breathing, coughing, laughing, yawning or other respiratory acts, is in all probability not angina pectoris.\*

G. NOCTURNAL ANGINAL ATTACKS. More than  $\frac{1}{2}$  of the patients in this series had frequent or occasional attacks of pain occurring at rest during the night (Table 3, part VI). The most common cause of such attacks seemed to be the assumption of the recumbent posture, a factor which was shown to be important in 11 patients. Exceptionally, nocturnal attacks were found to be related to spontaneous hypoglycemia, ectopic tachycardia, nightmares, cold sheets, or the exertion associated with paroxysmal dyspnea. Finally, there were 3 patients who had fairly frequent attacks at night and in whom a relationship to any of these precipitating factors could not be found. The mechanism of their nocturnal attacks remains unexplained. In addition there were 2 patients with angina who had nocturnal recumbent chest pain believed to be not anginal in nature, as each of them had an esophageal hiatal hernia.†

IV. Apparent Recovery from Angina Pectoris (Table 4). The findings cannot be regarded as quantitatively accurate because one does not know how many of the patients who seem to have recovered will in the future again have anginal attacks, and one is also ignorant of the number of patients who at present are still having attacks but who will cease having them in the future. Relatively little attention has been paid to the fact that certain individuals with undoubted angina pectoris

\* Aside from the 2 patients shown in Table 3, part V, there were 4 other individuals who had some of the features of angina pectoris but whose pain was related to respiratory movements. Since these subjects did not satisfactorily fill the criteria for the diagnosis of angina pectoris, they are not included in the present paper but are classified as "chest pain—cause unknown." They are being followed and thus far none of them has developed unquestionable evidence of disturbed coronary circulation.

† Since this report was written, a patient has been seen with nocturnal angina due to an additional factor—fatigue. This 67 year old male had had for 9 months attacks of "squeezing" pain just to the left of the sternum, with radiation to the left arm. The pain was induced by effort and lasted a few minutes only. He then developed typical clinical and electrocardiographic findings of "anterior" infarction. During convalescence he had several nocturnal attacks of pain, each of which occurred when he had become tired by remaining out of bed longer than usual during the preceding day. The mechanism whereby activity undertaken several hours previously induced pain is uncertain. I have observed other such cases, but since the data on this point are not complete, I am unable to state how frequently fatigue was a precipitating factor in the series of patients considered in this report.



do seem to recover. (Obviously, one cannot expect sclerotic coronary arteries to become normal. However, even though the disease in the vessels remains, the pain may cease either as the result of gradual death of the tissue from which the pain arises, or as the result of the development of collateral circulation.) As illustrations of apparent recovery the following cases may be cited.

TABLE 4.—APPARENT RECOVERY FROM ANGINA PECTORIS (ANALYSIS OF 12 CASES)\*

Conditions under which recovery occurred		No. of cases	Remarks	
Following coronary occlusion	.	4	Probable death of tissue responsible for painful stimulus	
Control of hypoglycemia	.	2	No evidence of structural cardiac disease; disturbance of function controlled by treatment	
	.			
	.			
Relief of anemia	.	1	Presumably either (1) development of collateral circulation, or (2) gradual replacement fibrosis of tissue giving rise to pain	
No apparent cause for recovery	.	5		
			* Recovery is assumed when no attacks have occurred for 1 year or longer, despite reasonably normal activity.	

\* Recovery is assumed when no attacks have occurred for 1 year or longer, despite reasonably normal activity.

CASE 4. A 55 year old male complained in 1934 of a squeezing, precordial pain brought on by rapid walking, and relieved within a few minutes by standing quietly. Physical examination was negative. Various laboratory studies revealed mild diabetes but no changes in the electrocardiogram were noted and the heart appeared to be normal when visualized by the fluoroscope. Walking briskly up 2 flights of stairs induced a typical attack. Thirty minutes later the same exercise taken shortly after nitroglycerine caused no pain. In 1936 the attacks were somewhat more frequent, appearing 2 to 3 times a week. The following year the condition was unchanged, but after an additional year the attacks had become very infrequent, occurring only once in every 3 or 4 months. During the next 3 years the patient remained completely free of anginal attacks. The electrocardiogram was normal throughout the entire course and the amount of activity was not significantly changed.

CASE 5. A white female, aged 68, stated that 5 years before she had had severe constrictive pain in both arms, coming on with effort. After several months she had an unusually violent attack lasting several hours, requiring hypodermics and hospitalization for relief. Since then the pain had been much milder but she had continued to have slight discomfort in both shoulders when she would undertake rapid walking. The blood pressure had ranged from 180 to 200 systolic prior to the severe attack of pain, but during recent years had been much lower, varying between 120 and 140. Examination revealed slight cardiac enlargement and numerous premature beats. Electrocardiograms showed frequent ventricular premature beats and inversion of the T waves in Leads I and IV. Under observation, nitroglycerine relieved the pain and inhibited its appearance during exercise. When she was seen 2½ years later she had been completely free of pain for the preceding 18 months, even though her activities had not been significantly modified.

When anginal attacks due solely to hypoglycemia, hyperthyroidism, paroxysmal tachycardia or to severe anemia disappear following adequate treatment of the causative factor, or when the seizures vanish after myocardial infarction, the mechanism seems reasonably clear. However, it is difficult to account for the improvement which occurs gradually in the absence of such conditions, except by assuming either gradual death of the tissue, with degeneration of the nerve fibers carrying the pain impulses, or the development of adequate collateral

circulation. In any case it should be emphasized that patients may recover from angina pectoris and that such recovery is probably not very unusual.

**The Occurrence of Chest Pain Due to Conditions Other Than Angina Pectoris in Patients Who Also have Chest Pain Due to Angina Pectoris** (See Table 5). The two most common conditions causing non-anginal pain were myocardial infarction and reflex disorders of the skeletal tissues, probably secondary to and an indirect result of the coronary disease. Since these conditions are closely allied to angina pectoris, they need not be discussed further. Even when these disorders are omitted, there were still 15 patients, or approximately  $\frac{1}{5}$  of the total number, who had two different causes of pain in the chest. The most common causes were spasticity and/or distention in the alimentary tract. The following cases are examples.

TABLE 5.—ANALYSIS OF CONDITIONS CAUSING NON-ANGINAL PAIN IN THE CHEST OR ARM IN 77 PATIENTS WITH ANGINA PECTORIS

Condition	No. of cases	%
Coronary thrombosis	35	45.4
Skeletal pain*	8	10.4
Spasticity of alimentary tract	6	7.8
Diseased gall-bladder	3	3.9
Hiatal hernia	2	2.9
Pulmonary infarction	2	2.6
Spasm of diaphragm	1	1.3
Cascade deformity of stomach	1	1.3
Total	55	75.4

\* Pain in muscles, joints, or bones. In these patients the pain was probably an indirect reflex result of the disease process in the heart.

CASE 6. A 48 year old man had a substernal cramping pain radiating to the left shoulder and down the left arm as far as the elbow. This was often induced by excitement or exertion and lasted a few minutes. However, he had had numerous—i. e., several score—attacks of similar pain occurring with no obvious precipitating factor and lasting from several hours to as long as 2 days. Nitroglycerine relieved the pain of short duration but had little effect during the longer seizures. Examination revealed moderate cardiac enlargement and signs of aortic stenosis. Under observation, exertion induced the discomfort, but the same exercise did not induce it when carried out immediately after nitroglycerine. Fluoroscopic examination revealed marked spasm at the lower end of the esophagus. This was not found following atropinization, which benefited markedly the attacks of long duration but did not materially effect the pain brought on by effort.

This individual's condition was very similar to that of Edekin's patient, who likewise had chest pain due to both angina pectoris and esophageal spasm. Wolferth and Edeiken<sup>23</sup> emphasized the frequency of esophageal spasm, pointing out that 53 cases had been observed in 2 years and that 47 of these had pain resembling in some degree the pain of angina pectoris but lacking the characteristic relationship to exertion. A number of patients had pain in the lower left chest due to distention of the stomach or colon. Some of these were able to differentiate this from the anginal pain induced by effort; others could make no such distinction.

CASE 7. A 43 year old man had "sour stomach" and belching associated with slight substernal discomfort for approximately 15 years. For the past month he had complained of a gnawing feeling in the epigastrium, a pressure sensation in the chest, and an ache in the jaw. These several sensations were brought on by exertion and disappeared in 3 to 5 minutes with rest. The discomfort would also come shortly after meals and he thought that it was partially relieved by belching or expulsion of flatus. He was unable to differentiate between the discomfort associated with abdominal distention and that brought on by effort. Physical examination revealed minimal hypertension and slight cardiac enlargement. Roentgen ray studies of the gall bladder were negative. Gastro-intestinal Roentgen rays revealed elevation of the splenic flexure and questionable pylorospasm. Resting electrocardiogram was normal except for minimal (0.5 mm.) elevation of the S-T segment in Lead IV. Exercise induced his discomfort, and following the exertion there was a depression of 1 to 1.5 mm. in the S-T segments in Leads II, III and IV. Following inflation of the colon with air, only three-fourths as much exercise was required to bring on the same pain and the same electrocardiographic changes. Similar exertion undertaken after nitroglycerine induced no discomfort until 50% more exertion had been taken.

When, as occasionally occurs, gall-bladder disease induces pain referred to the left chest anteriorly rather than to the right side of the chest posteriorly, it may cause considerable confusion. The situation becomes doubly complex when a patient of this type likewise has angina pectoris. Such was the case in 3 patients in this series. (It seems probable that more than 3 of the 77 patients had gall-bladder disease. However, cholecystograms were not made routinely and this discussion is concerned only with the 3 patients in whom we were able to demonstrate that gall-bladder disease was causing pain in the chest in addition to pain in the chest due to angina pectoris.) An illustration of chest pain due to the two disorders is as follows:

CASE 8. A 69 year old white male was first seen in 1932 with typical anginal attacks brought on by effort and relieved by rest. In 1935 he had a severe and almost fatal myocardial infarction. Following this the anginal attacks ceased for 3 years, but in 1938 he again began having pain brought on by effort and radiating from the substernal region to the right shoulder. In addition he began to have attacks of pain in the right upper quadrant with occasional radiation to the right scapular region, but with usual radiation to the left substernal and precordial regions. This pain bore superficial resemblance to the anginal pains, but he was able to distinguish the two as regards location and duration. The pain associated with gall-bladder disease lasted for hours and was not affected by nitroglycerine. It sometimes followed eating and was often benefited by belching or vomiting. In addition, he had pain on moving the right shoulder. Roentgen rays revealed hypertrophic arthritis in the right shoulder joint in addition to stones in the gall-bladder. In this individual we have at least 4 different painful conditions in and about the chest. These were: angina pectoris, myocardial infarction, disease of the gall-bladder, and arthritis of the right shoulder joint. Whether this arthritis was of entirely independent nature or whether the development of the arthritis was reflexly conditioned by the attacks of angina with secondary trophic changes in the joint is uncertain.

Numerous other instances could be cited of patients who had chest pain due to more than one cause. However, the other disorders which produce pain in the chest will be discussed in future publications, and the cases which have been mentioned are sufficient to justify emphasis.

sizing the fact that a large percentage of the individuals with angina pectoris have pain in the chest due to independent disorders. It therefore is apparent that one can readily overlook the presence of angina pectoris unless a most careful history is taken and unless the patients are studied completely, particularly as regards the observation of the response to exercise.

TABLE 6.—THE RELATIVE VALUE OF VARIOUS PROCEDURES IN THE DIAGNOSIS OF ANGINA PECTORIS

Positive results	No. of cases	No. of cases in which method was employed	OF ANGINA PECTORIS			
			History (of pain* on effort)	Physical examination†	Electrocardiograms:	At rest.
52.2	36	69	69	69	69	69
60.0	6	10	10	10	10	10
60.0	36	60	60	60	60	60
89.2	33	37	37	37	37	37

\* The findings by these methods were often of great value in regard to the specific diagnosis of angina pectoris.

† The findings by these methods were rarely of value in regard to a diagnosis of angina pectoris, but were often of great value in the diagnosis of cardiac disease.

TABLE 7.—SUMMARY OF THE MOST IMPORTANT CLINICAL FEATURES IN THE DIAGNOSIS OF ANGINA PECTORIS

II. Factors of Negative Value (Their Presence Tending to Exclude Angina Pectoris)†			
1. Pain limited to periaxillary or abdominal regions	2. Duration of less than 1 minute	3. Lacerating or throbbing pain	4. Aggravation by breathing, coughing, swallowing, sitting or standing

\* These clinical features present in 90% or more of the patients.  
† These features either absent in angina pectoris, or, if present, due to associated disorders.

**The Relative Value of Various Procedures in the Diagnosis of Angina Pectoris** (See Tables 6 and 7). Obviously, the physical and roentgenographic examinations are of no direct value in diagnosis of angina pectoris. However, by furnishing evidence of cardiovascular disease in something like  $\frac{2}{3}$  of the patients, these procedures are of considerable indirect value. The same generalization applies to electrocardiograms taken in resting subjects. Somewhat more than half of the patients displayed changes which, when considered in relation to the age and to the blood pressure of the patient, could be considered as definitely abnormal. In a majority of instances such electrocardiographic changes simply pointed toward some myocardial disorder and were not in any way characteristic of angina pectoris.

When electrocardiograms are taken following muscular effort, changes of the S-T segments and of the T waves of such a nature as to be specifically suggestive of the presence of angina pectoris may be encountered in 50 to 60% of the patients. This was true not only in the small series of my own subjects in which the procedure was carried

out, but in a much larger group of patients which has been studied by others. Thus, Twiss and Sokolow<sup>26</sup> have recently summarized the literature on this point and have reported observations on 66 patients with angina and 100 control subjects. Fifty-six per cent of their patients with angina pectoris developed abnormal changes in electrocardiograms, *i. e.*, changes of greater degree than were encountered in the control series. Similar findings have been reported by investigators<sup>27,28</sup> who have studied electrocardiographic changes induced by breathing 10% oxygen. Here again 50 to 60% of anginal patients have shown changes in the T waves and S-T segments of greater magnitude than those occurring in the control subjects.

Although, in general, the results of attempts aimed at improving the accuracy of the diagnosis of angina pectoris by taking electrocardiograms under various conditions have been disappointing, there are occasional patients with confusing histories in whom records made before and after effort may be of considerable diagnostic significance. An example follows:

CASE 9. A 71 year old female complained of precordial discomfort radiating to the right shoulder and the left arm and lasting from a few minutes to half an hour. Many of her attacks appeared when she was lying down and there would be some relief within a few minutes after sitting up. In addition, she occasionally had attacks coming on during exercise. She stated that the pain was at times aching in character and at others stabbing. She had diabetes and a non-functioning gall-bladder, as shown by cholecystograms. Electrocardiograms taken at rest were normal for a woman of her age, but following exertion there was a well marked inversion of the T waves in all leads.

Although electrocardiograms may, in certain patients, be of great value in the diagnosis of angina pectoris, too much reliance on them may be misleading. Thus, the records of one of the patients already mentioned (Case 5) in whom the attacks of angina ceased entirely, taken after she had been free from attacks for more than a year, were identical with those taken when she was having frequent seizures. Since it is the people who are having the attacks of pain rather than those who show electrocardiographic changes who are in danger of sudden death, serious errors in judgment may be made by placing too much weight on electrocardiograms in contradistinction to the clinical picture.

The methods of diagnosis thus far discussed are the objective ones. Those which rely on subjective information may now be considered. Two features which were of the greatest value in diagnosis were (1) the story of the relationship of the pain to effort which was obtained in approximately 90% of the patients, and (2) observation of the effects of exercise with and without nitroglycerine, which gave approximately an equally high percentage of positive results. Obviously these procedures often had to be carried out with the greatest of care. Some of the patients gave very confusing stories, and this was particularly true of those who had more than one cause of pain in the chest. At times history obtained initially would indicate no relationship between the pain and effort when subsequent careful questioning would point

toward such a relationship. Certain other patients were so interested in the relation of the pain to the gastro-intestinal tract, and were so convinced that "indigestion" was the source of the trouble that they had paid little attention to the effect of effort, and did not recall attacks associated with exertion until questioned in considerable detail. It was under such circumstances and also in patients with actual or impending congestive failure, who became so dyspneic with effort that they paid little attention to the coexistent pain, that observation of the response to exercise was particularly valuable, because it served as a check against the possibility that the story of the relation to effort had been planted in the patient's mind by the physician's questions. Since pain induced by exertion occasionally occurs in conditions other than angina pectoris, the most reliable results were obtained by comparing the response to effort with that after nitroglycerine. In order to avoid the effect of suggestion, a third period of exercise undertaken following the administration of a placebo was usually employed. In emotionally unstable patients, it was often necessary to carry out this procedure a number of times before one could be satisfied with the result. However, when proper precautions were observed, the study of the effect of nitroglycerine on the exercise tolerance was found to be a valuable aid to the diagnosis of angina pectoris.

In Table 7 are summarized those clinical features which seem to be particularly helpful in diagnosis. Aside from the story and from the observation of the effect of nitroglycerine on the tolerance to exercise, the most helpful positive features were the duration of the pain and the localization in either the precordial or substernal regions. Each of these findings was present in about 90% of the patients. In addition, there were certain features which appeared to be of considerable negative value in that they occurred very rarely or not at all, or when present were due to associated disorders. These features, which occur so rarely in patients with angina pectoris as to constitute evidence against the disorder, are: (1) pain limited to the periaxillary, or abdominal regions; (2) duration of the pain of only a few seconds; (3) a throbbing or lancinating character of the pain; and (4) discomfort which was aggravated by swallowing, breathing, coughing or the sitting or standing posture.

There are certain findings which are ordinarily stressed in the diagnosis of angina pectoris and which occurred in about half of our patients, but which appeared in this series less frequently than in some of the other reports in the literature, and which therefore would seem to be of less diagnostic value than is generally believed (Table 8). These included (1) pain limited to the substernal region, (2) pain of constrictive quality, and (3) electrocardiographic changes either at rest or after exercise. On the other hand, there were certain features which, although less common than the ones just mentioned, still were more common in this series than one would think, from the writings on angina pectoris in the literature. These included pain of mild character, discomfort occurring at night or aggravated by the recumbent position, and distress which tended to be benefited by eating. It is

be added to the classic list of exertion, eating, emotion and cold as important precipitating causes of the attacks (Table 8).

TABLE 8.—SOME COMMON MISCONCEPTIONS IN REGARD TO ANGINA PECTORIS

II. Features Which Are More Common Than Usually Believed†	
1. Mildness of pain	1. Location in substernal region or in arms
2. Aggravation by recumbent posture	2. Constrictive quality of pain
3. Beneficial effect of eating	3. Abnormal electrocardiograms at rest
4. Nocturnal attacks	4. Abnormal degree of change in S-T segments or T waves after effort
5. Frequency of chest pain due to other diseases in patients who also have chest pain due to angina pectoris	* These features present in 40 to 60% of the patients.
	† These features present in 15 to 50% of the patients.

**Palpitation in Relation to Angina Pectoris.** The data in our study on this point are not summarized because they are not complete, as all of the patients were not questioned in detail about this symptom. My general impression is that palpitation is an exceptional symptom during anginal attacks, and that its occurrence should immediately lead to the suspicion that the pain complained of is due to some other cause such as cardiac neurosis. However, there are certain conditions under which palpitation commonly occurs during anginal seizures. The most common cause is frequent premature beats. Rarely anginal attacks are precipitated by ectopic tachycardia, either auricular fibrillation or paroxysmal tachycardia. Such a patient may have pain only during the seizures. The following case is illustrative:

CASE 10. A 58 year old man was seen because of palpitation. He gave a typical story of auricular tachycardia, the attacks having been present for approximately 30 years. During the seizures he had severe palpitation, weakness, and marked apprehension, but had never had pain. Opportunity to confirm the diagnosis by electrocardiogram did not occur, but on two occasions the attacks were stopped by pressure on the eyeballs, and in all respects the seizures were typical of paroxysmal auricular tachycardia. Three years later, at the age of 61, he began to have protracted and subterminal constrictive pain during the seizures. Following one rather prolonged attack he developed cerebral embolism with hemiplegia. Several years later he began to have occasional attacks of angina on walking and now, for the first time, had pain independent of the attacks of tachycardia. He expired suddenly at the age of 67, while in his usual health.

Aside from disturbances of cardiac rhythm, palpitation may occur in anginal attacks brought about by hypoglycemia. This symptom was present in 1 of the 2 patients in this series in whom hypoglycemia was the sole cause of the attacks and occurred in 8 of the 12 patients who had anginal seizures due to hypoglycemia in addition to attacks occurring with effort. The palpitation was usually absent in the seizures brought on by exertion and present in the attacks occurring at rest, due to glucose deficiency.

Thyrototoxicosis may lead to anginal attacks accompanied by palpitation, and a rarer cause is a condition which is not illustrated in this series, namely, paraganglioma of the adrenal glands with intermittent

hypertepinephrinemia. However, as a general rule, it may be said that when a patient with undoubted anginal attacks has palpitation during the seizures in the absence of a well-marked disturbance of the rate or of the rhythm of the heart, one should suspect hypoglycemia as the cause, and particularly so if the attacks occur in the resting state.

**Anginal Attacks Occurring Independently of Muscular Effort.** *Individuals Who Had Never Had Pain in Relationship to Exertion.* There were only 7 such subjects out of the 77 patients in the series. One of these individuals was almost bedridden because of thrombo-angitis obliterans. Two other subjects in the group led unusually sedentary lives and indulged in practically no exercise other than walking a few yards at a time. One patient had paroxysmal auricular fibrillation and another had paroxysmal tachycardia. The pain in these 2 subjects appeared only during the seizures of ectopic rhythm, but neither of them engaged in much exercise. The remaining 2 patients led active lives and were subjected to strenuous exercise under observation, but had no pain. These 2 subjects had angina only when hypoglycemia was present. One of them has been reported in a previous publication<sup>15</sup> and the other is described as Case 3 in this communication. It thus appears that when a patient is suspected of having angina pectoris and yet gives no story of any relationship of the seizures to physical exertion, one should suspect ectopic tachycardia, glucose deficiency, or that the individual is leading an unusually restricted life. Most of the subjects who fall into the latter group can be induced to have an attack if sufficient physical effort is undertaken. However, in the case of individuals with ectopic tachycardia and with hypoglycemia, anginal attacks may occur in spite of the fact that the same individuals under different circumstances may undertake severe exertion and remain entirely free of pain.

*Individuals With Frequent or Occasional Angina of Effort and With Frequent Attacks Occurring in the Absence of Effort.* Such patients include a fairly large percentage of all individuals with angina pectoris, and since cases of this type are the ones which are likely to offer the greatest difficulty in diagnosis, it may be worth while to re-summarize the factors which may induce attacks at rest. Most of the importance of the recumbent posture has been stressed. Most patients who have attacks during recumbency have seizures readily provoked by mild exercise. However, occasionally a patient may have relative freedom from attacks during effort and yet have fairly frequent seizures coming on while lying down. In such a patient, unless a clear-cut story can be obtained of attacks during exertion, it may be necessary to have the patient undertake exercise under observation in order to establish a diagnosis. There are a good many patients who have occasional attacks during effort and who have seizures occurring at rest without any obvious precipitating factor, in whom careful analysis will demonstrate that the attacks coming on at rest are due to glucose deficiency. The following patient is an example:



CASE 11. A 44 year old, very obese white male had, while walking at a rapid rate against the wind in very cold weather, an attack of discomfort consisting of a dull heavy sensation over the entire anterior chest. This pain lasted for about 30 minutes. Several months later he had a series of similar attacks, lasting 1 to 2 hours, occurring at rest, the last of the series being more severe, enduring for about 8 hours, and being associated with the typical manifestations of myocardial infarction. Since that time he had had numerous short seizures of similar but milder pain occurring without any relationship to effort, but his activities had been markedly restricted. These attacks had usually come 3 to 5 hours after the preceding meal. For several years he had been promptly relieved by eating. The only important finding on physical examination was marked obesity. Blood pressure was 120/84. Fluoroscopic examination revealed suggestive hypertrophy of the left ventricle. Electrocardiograms displayed moderate downward displacement of the S-T segment in Lead II, slight displacement in Lead III, and a diphasic T 4. Following the ingestion of glucose his blood sugar was 220 mg. per 100 cc. after 1 hour and 50 mg. per 100 cc. in 4 hours. Further inquiry revealed that in recent weeks the patient's usual attacks of weakness and nervousness, which had occurred several hours after meals for years, and were relieved by eating, had been associated with the pain in the chest.

The importance of large meals and of emotional disturbances in causing anginal attacks at rest has already been stressed, and the occasional patient in whom attacks occur as the result of abdominal distention independently of meals has been mentioned. It should be emphasized that patients with angina due to diseases of the aortic valve are considerably more prone to attacks at rest than are patients with angina as the result of coronary sclerosis. An occasional individual will have anginal attacks occurring during the night and brought about by unpleasant dreams. There was only one such case in the present series. She also had attacks with exertion, but these were less common than those associated with nightmares. As patients develop either congestive failure or intermittent claudication, seizures previously appearing with effort may disappear because the subject is no longer able to undertake as much muscular effort as previously. After a time such individuals may begin to have attacks at rest. This occurred in one patient in this series and may have taken place in another. When the several precipitating factors which have been mentioned have been excluded, there still remain certain patients who have attacks at rest in the absence of any known cause. Possibly in such individuals measurements of blood pressure and other circulatory functions might yield information concerning the mysterious mechanisms concerned in the seizures.

*Individuals With Attacks of Angina at Rest as a Prelude to Myocardial Infarction.* The conditions which have thus far been mentioned as causes of angina without exertion usually lead to only occasional attacks. When a patient begins to have frequent attacks at rest, and particularly if the attacks are unusually severe and last for an hour or more, the chances are strong that the patient will soon develop a myocardial infarction. In a fairly large percentage of cases the onset of infarction is preceded by such a sequence of events. Occasionally,

such patients with *status anginosus* may die suddenly without having ever developed the infarction. It is probable that these "pre-infarction" seizures are the most common cause of resting angina, particularly if the attacks are severe. However, this type of angina has not been considered in the present publication because it is closely allied to myocardial infarction and may be more properly considered in a future report dealing with that disorder.

It must be granted that many patients with typical angina pectoris have occasional attacks at rest, and that in exceptional circumstances all of the attacks in a given individual may occur in the resting state. However, the recognition of this fact should not lead the physician into a state of being ready to diagnose angina in the absence of the story of muscular effort. Pain simulating angina pectoris in location and in character, but without a relationship to effort, is of common occurrence, and in the majority of instances the pain is not really that of angina pectoris at all but is dependent on a disorder of organs other than the heart. One should therefore demand rather conclusive evidence before being willing to place the ominous label of angina pectoris on a patient who does not have attacks with exertion. This fearful disease may be simulated by many others. The differentiation, while often difficult, is usually possible, and it is primarily with the problem of such differentiation that future reports in this series will deal.

**Summary.** An analysis of 77 patients with angina pectoris has been made, with particular reference to the characteristics of the pain and its relationship to various body functions.

The pain was felt in the substernal location in only about  $\frac{2}{3}$  the patients. Pain entirely limited to the periaxillary or abdominal regions did not occur in any case.

The duration of the pain was usually a few minutes only, rarely longer than  $\frac{1}{2}$  hour. No patient had pain lasting for a few seconds only.

Pain of great intensity was exceptional, the discomfort being mild or minimal in more than  $\frac{3}{4}$  the patients.

The discomfort was constrictive or heavy in character in only about 50% of the cases. Frequently, the pain was of an aching quality; burning discomfort was occasionally found; while lancinating pain was encountered in only one subject.

In addition to the generally recognized "trigger" factors of exertion, eating, emotion and cold, the recumbent posture and glucose deficiency were found to be common precipitating causes of the seizures. In exceptional patients, anginal attacks with typical electrocardiographic changes may be induced by spontaneous hypoglycemia in patients who have no seizures with severe effort and no evidence of structural cardiac disease. The act of eating may precipitate anginal attacks in certain patients and may prevent the attacks in other subjects.

Pain induced by the sitting or standing position or aggravated by breathing, coughing or swallowing can usually be safely ascribed to disorders other than angina pectoris.

In the diagnosis of angina pectoris the most important features are: (1) the history of relationship to effort, (2) the short duration of the pain, (3) the demonstration that the amount of muscular effort required to induce the pain is increased by nitroglycerine.

A large percentage of patients with angina pectoris also suffer from chest pain due to other disorders. Such disorders may either be related to angina pectoris (as in the case of myocardial infarction and reflex disturbances of the skeletal system) or unrelated to it (as in the case of gall-bladder disease, hiatal hernia, esophageal spasm, and so forth). Because of the frequent coexistence of two causes of chest pain, one of them may be overlooked unless unusual care is employed in obtaining the history.

Occasional patients—about 10% in this series—may have anginal attacks which have never been related to effort. Among the causes of such attacks are: status anginosus ("coronary insufficiency," "pre-infarction angina") ectopic tachycardia, spontaneous hypoglycemia, and conditions such as intermittent claudication, congestive failure and undue anxiety about the cardiac condition, which induce the patient to lead an unusually sedentary life. It is in this group of patients that the greatest diagnostic difficulty is likely to be encountered.

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# INFARCTION OF THE LATERAL WALL OF THE LEFT VENTRICLE PATHOLOGIC AND ELECTROCARDIOGRAPHIC STUDY

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The electrocardiographic (ECG) pattern of infarction of the lateral wall of the left ventricle (mid-ventricular infarction) was first described by Wood, Wolfarth and Bellet.<sup>14</sup> They described the following ECG changes as typical: (1) Depression of the RST segment in Lead IV. (2) Depression of the RST segment in Leads I and II. This they stated was commonly, though not universally, present. (3) Absence of characteristic abnormalities in Lead III. (4) High incidence of auricular fibrillation.

The resemblance to digitalis effect in the S-T segment was also noted. One typical, uncomplicated autopsy case was reported. Left circumflex artery thrombosis was usually found to be responsible for the infarction.

Since their paper in 1938, the only report has been that of Johnston, Rosenbaum and Willson<sup>15</sup> who write that "Antero-lateral infarction gives rise to characteristic changes in leads from the left side of the precordium and in Lead I. Postero-lateral infarcts often produce abnormally large Q waves in the leads from the extreme left side of the precordium and give rise to inverted T waves in Lead I and prominent Q waves in Leads II and III. When antero-septal infarction is followed by postero-lateral infarction, there is a tendency for the QRS changes produced by the former to disappear or become less typical. Under these circumstances R waves may reappear in leads from the right side of the precordium and typical or semicharacteristic modifications of the ventricular complexes of the fifth and sixth precordial leads appear for the first time."

With the object of inquiring further into the incidence and diagnostic criteria of this type of infarction, 106 postmortem cases of myocardial infarction were reviewed. The entire group of cases (Table I) was subdivided, depending on the location of the infarct.

TABLE I.—POSTMORTEM CASES OF MYOCARDIAL INFARCTION SUBDIVIDED ACCORDING TO LOCATION OF INFARCT

Location	No.	%
Left lateral wall	19	17.9
Right lateral wall	1	0.9
Posterior and basal	26	25.5
Anterior and apical	56	54.9

We have also added 1 case of contusion of the lateral wall of the left ventricle by a bullet wound, making a total of 107 cases. This

incidence of left lateral infarction is in accord with that of Barnes,<sup>1</sup> who found 8 cases in 49 cases of myocardial infarction (16.3%).

Barnes<sup>1</sup> in 1932 stated that in a heart with an unusually large left circumflex artery or with neighboring arteries congenitally small or obstructed by disease, occlusion of this vessel results in a more extensive infarction, involving the anterior or posterior wall of the left ventricle, producing complex electrocardiographic patterns.

We have divided our cases of lateral infarction into the following groups: (1) Recent pure lateral infarcts with "typical" electrocardiograms (4 cases). (2) Recent pure lateral infarcts with atypical electrocardiograms (5 cases). (3) Recent lateral infarcts combined with infarction of some other part of the myocardium (3 cases). (4) Remote lateral infarcts (7 cases).

In every case a postmortem examination was performed and an ECG had been taken just before death in the cases of recent infarction. The location of the recent infarcts in the first 9 cases are illustrated in Figure 1, together with the coronary arteries involved. Figure 2 shows the typical ECG findings in the first 4 cases. Figure 3 comprises the ECG of the atypical cases.

#### Group 1. Cases With "Typical" ECG. This group comprises those

cases of recent infarction of the lateral wall which showed ECG changes similar to those described as typical of this type of infarction by Wood, Wolferth and Bellet.<sup>14</sup> These patients had not received digitalis at the time the ECG showing the typical changes were taken. Of these cases, 3 had remote infarcts in addition to the recent infarction. In 1 case the infarct was extensive and occupied a part of the anterior wall; in another the infarct extended slightly into the posterior wall. The left circumflex coronary artery was occluded by a recent thrombosis in 1 case; in 2 cases the left coronary artery was thrombosed near its origin; in another case only sclerosis of these arteries was present.

In all of our 4 cases, the S-T segment was depressed in the chest leads. These cases showed a slight depression of the S-T segment in the limb leads. Two of these cases showed a slight elevation of the S-T segment in Lead I. In only 1 case were the T waves upright in all leads. Contrary to the findings of Wood, Wolferth and Bellet,<sup>14</sup> the QRS complex showed some notching in 2 of our cases.

#### Case Studies. Case 1. Female, aged 61. *Clinical Diagnosis.* Myocardial infarction; generalized arteriosclerosis. Five years previously, patient was awakened from sleep by a dull aching pain in the left chest radiating to the left shoulder and arm. Known high blood pressure for 10 years. Twenty-four hours before admission, sudden seizure of pain beginning over the precordium, radiating to the left shoulder and down to the fingers. Not relieved by nitroglycerin. This persisted until 4 hours before admission when it suddenly increased.

*Physical Findings.* Cyanosis, distention of neck veins, shallow respiration, slight accentuation of A<sub>2</sub>, blowing systolic murmur at apex was noted. A few coarse rales over both bases; later, loud moist rales. White blood cell count 10,000; sedimentation rate 0.5 mm. per minute. Temperature rose to 39.2° C. on the 2nd hospital day. On 5th hospital day there was a sudden return of agonizing pain. Cheyne-Stokes respiration, diaphoresis, cyanosis and a fall

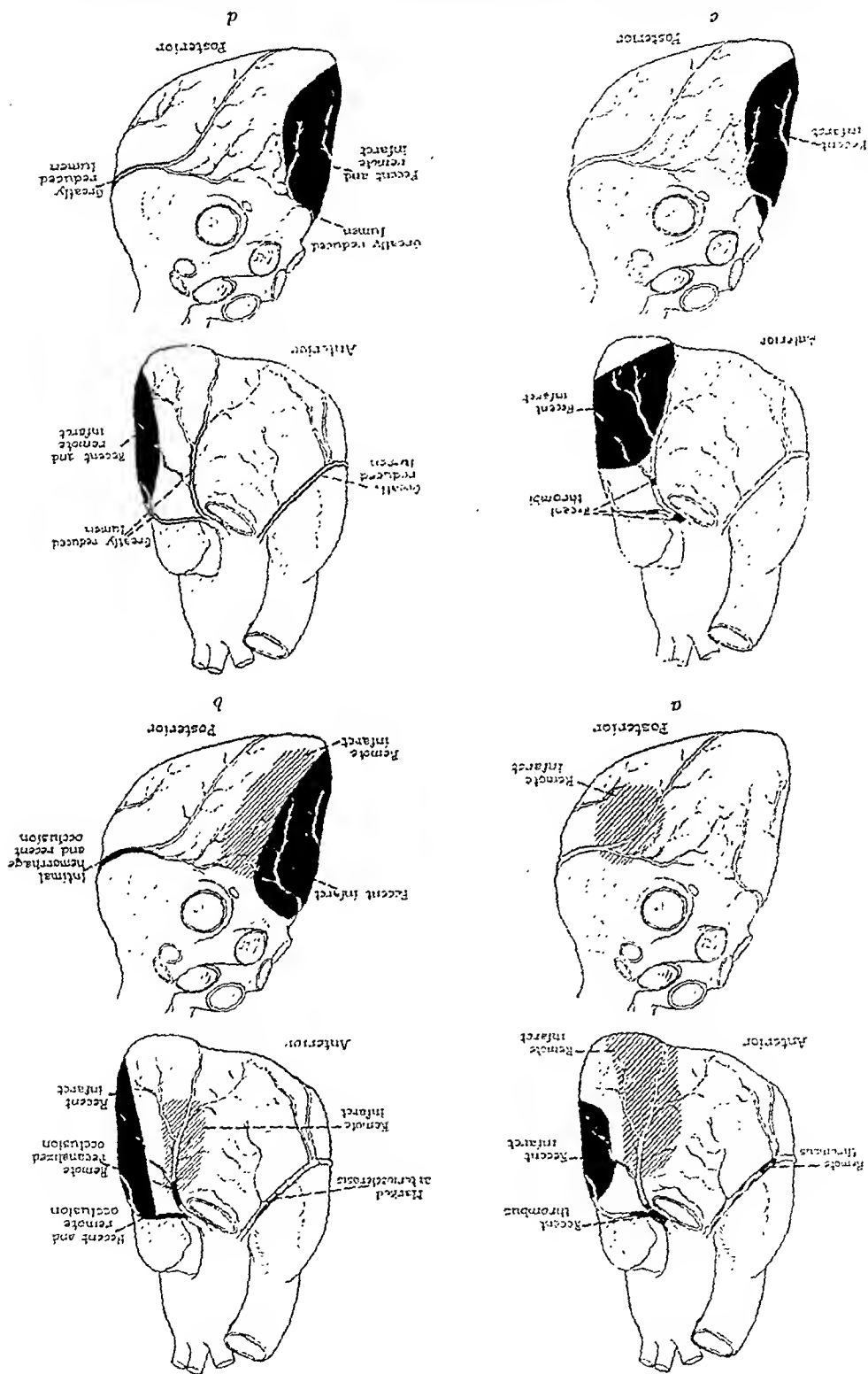


FIG. 1 (a-d).—Diagrams of hearts, showing the location of recent pure infarcts of the lateral wall of the left ventricle and the vessels occluded by thrombi, or the lumina reduced by atherosclerosis.

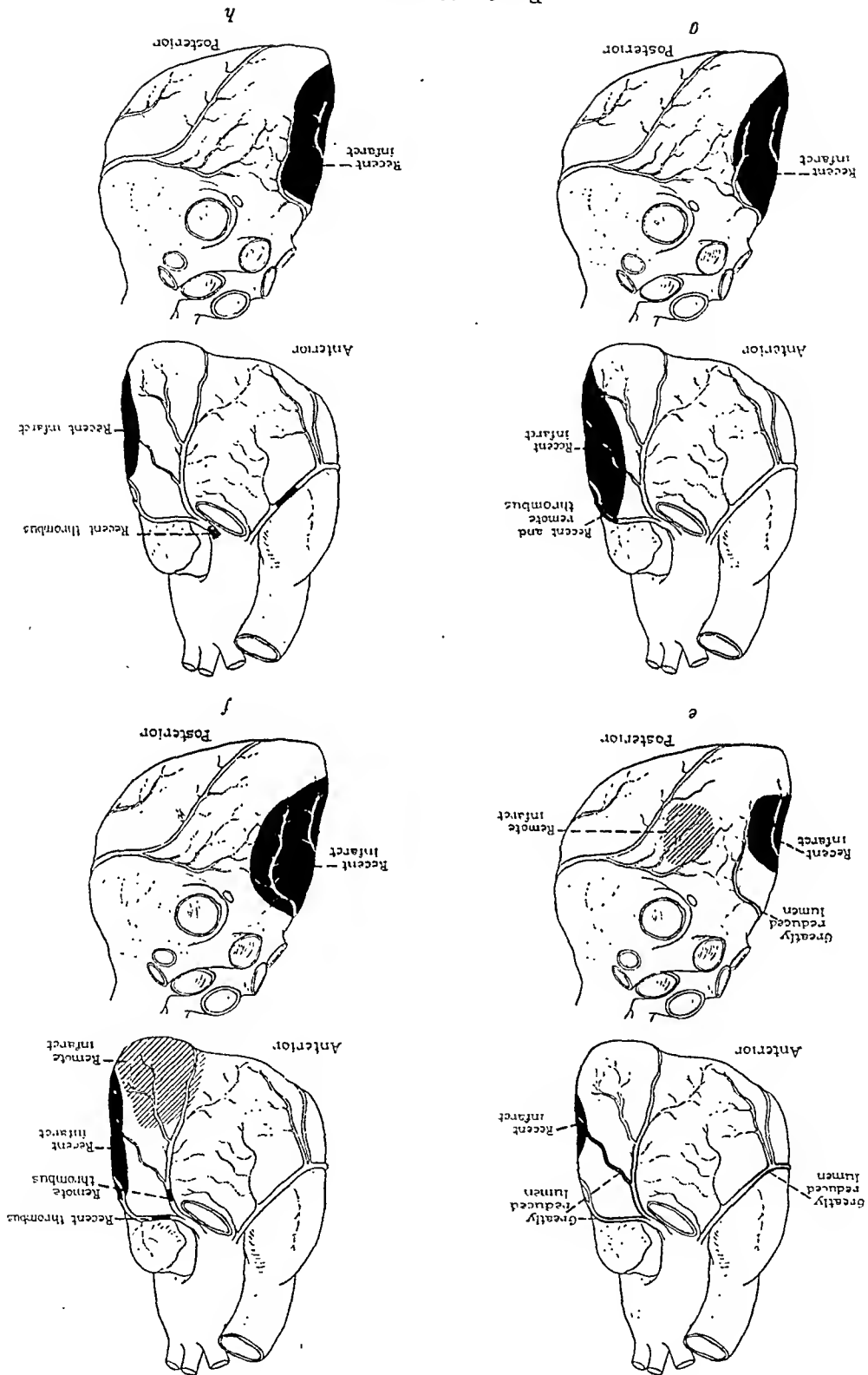


FIG. 1.—(Continued.)

of blood pressure to 90/70. On the 7th hospital day another severe attack of pulmonary edema and pain occurred, followed by sudden death. The patient had digitals on the 4th and 5th hospital days after the first and diagnostic electrocardiogram.

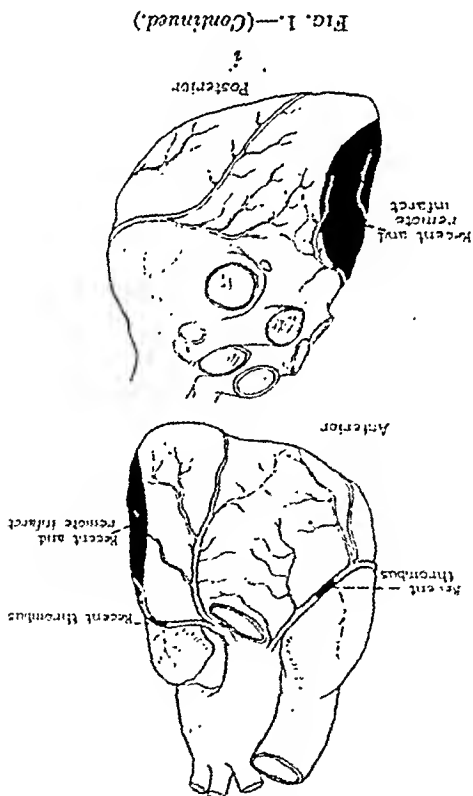


FIG. 1.—(Continued.)

*Pathologic Findings.* Recent infarct of the lateral wall of the left ventricle. Remote infarcts of the posterior and anterior walls of the left ventricle. Marked coronary sclerosis and stenosis. Recent thrombosis of the circumflex branch of the left coronary artery with extension of thrombus into left main coronary artery. The interventricular septum was involved by a remote infarct of the posterior wall of the left ventricle. Cardiac hypertrophy and dilatation (400 gm.) (Fig. 1, a). ECG, 1/30/40: A-V nodal rhythm, rate 75 per minute; abnormal left axis deviation; depression of S-T<sub>2,3,4</sub> (Fig. 2, a). 2/5/40 (day preceding death): S-T in 4R. Case 2. Male, age 57. *Clinical Diagnosis.* Myocardial infarction, remote and recent; generalized arteriosclerosis and hypertension; bronchopneumonia. Two previous admissions for bronchopneumonia and for right hemothorax. Hemiplegia 3 years previously. One week before admission a severe attack of nocturnal dyspnea with substernal oppression. Four days before admission a similar attack and the night before admission a third attack which was still more severe.

*Physical Findings.* Respiratory distress, diffuse apex beat, slight enlargement of the heart to the left, soft blowing, high-pitched systolic murmur following first sound at apex. Blood pressure 150/110; elevated venous pressure. White blood cell count 10,000. Type 23 pneumococci in the sputum. Increased sedimentation rate. Forty-eight hours after admission the patient had a severe pulmonary edema and died 36 hours later. ECG was taken on day of admission previous to digitalization.



*Pathologic Findings.* Recent infarct of the lateral wall of the left ventricle. Remote infarct of the anterior and posterior wall of the left ventricle. Compression of right coronary artery with some intramural hemorrhage and lymphocytic infiltration. Marked coronary arteriosclerosis. Hypertrophy and dilatation (500 gm.) (Fig. 1, b).

*ECG:* Regular sinus rhythm, rate of heart 114 per minute; right bundle branch block; depression of S-T<sub>1</sub> (Fig. 2, b).

*Case 3.* Male, age 78. *Clinical Diagnosis.* Parkinsonism; myocardial infarction. He had encephalitis in 1920 followed by Parkinsonism; angina for 3 weeks and a constant substernal pain with weakness for 12 hours before admission.

*Physical Findings.* Severe distress, peripheral cyanosis and cold extremities, a few basal rales. Heart normal in size and in sounds. Blood pressure 130/110. White blood cell count 14,500. Accelerated sedimentation rate. The day after admission auricular fibrillation began and the patient was digitalized. He suddenly expired on the 6th hospital day. The first and diagnostic electrocardiogram was taken on the day of admission and before digitalis had been given.

*Pathologic Findings.* Recent infarct of the left ventricle, most marked over the left lateral wall. The infarction of the left ventricle anteriorly was subendocardial and did not extend through the epicardium (Fig. 1, c).

*ECG, 1/4/43:* Regular sinus rhythm, rate 115 per minute. T<sub>1</sub> negative, S-T<sub>2,3,4,5</sub> depressed, frequent ventricular extrasystoles (Fig. 2, c). 1/7/43: Paroxysmal auricular fibrillation.

*Case 4.* Female, age 69. *Clinical Diagnosis.* Coronary thrombosis; arteriosclerosis; diabetes mellitus.

Complaint of precordial pain for 2 years. Persistent pain in chest and left arm for 2 weeks. Known hypertension for 8 years. On the day before admission, the patient became irrational, then comatose. The sputum for the last 3 days was grossly bloody and she vomited large amounts of blood.

*Physical Findings.* Ashen cyanosis, coma, shallow breathing, moist rales in both bases, systolic murmur to left of sternum in fourth intercostal space. Protodiastolic gallop. White blood cell count 8950; sedimentation rate not increased. Died in 1st hospital day. No digitalis therapy.

*Pathologic Findings.* Recent and remote infarction of lateral and basal wall of left ventricle. Coarse fibrous scar in interventricular septum. Fibrous obliteration of pericardial cavity. Severe coronary arteriosclerosis and stenosis. Lumen of left circumflex coronary artery greatly reduced. Hypertrophy and dilatation of heart (425 gm.) (Fig. 1, d).

*ECG:* A slight elevation of S-T segment in Lead I with the T wave inverted in the same lead. In both chest leads there was an abnormal depression of the S-T segments, proportionally greater in 4R (Fig. 2, d).

## Group II. Cases With Atypical ECG. This group comprises Cases 5

to 9 inclusive. The infarcts all involved the mid-ventricular region of the left ventricle. In 3 cases the left circumflex artery was obliterated by recent thrombosis; in 1 case the left coronary artery was occluded by a thrombus at its origin; in 1 case only sclerosis was present in the coronary arteries. In 2 cases there was occlusion of one of the other coronary arteries. Wood, Wolferth and Bellet<sup>14</sup> in discussing their atypical cases, observed that the ECG could be modified by the extension of the infarct into the anterior or posterior walls, so producing the atypical ECG findings. Two of our cases showed changes corresponding to a recent posterior and basal infarct and 1 case suggested an old posterior and basal infarct. In the other 2 cases, fibrillation was present but 1 of these had a depression of the S-T

segment in the chest lead which might have been more pronounced if pericarditis had not been present.

CASE 5. Female, age 68. *Clinical Diagnosis.* Arteriosclerosis; hypertension; coronary occlusion; cerebral hemorrhage and multiple infarction of the brain. Two previous admissions, the one for a surgical condition and the other for hypertensive heart disease and cardiac failure. The patient returned to the Out-patient Department after 1 month, complaining of pain and shortness of breath. On the same evening she became stuporous and developed left hemiplegia.

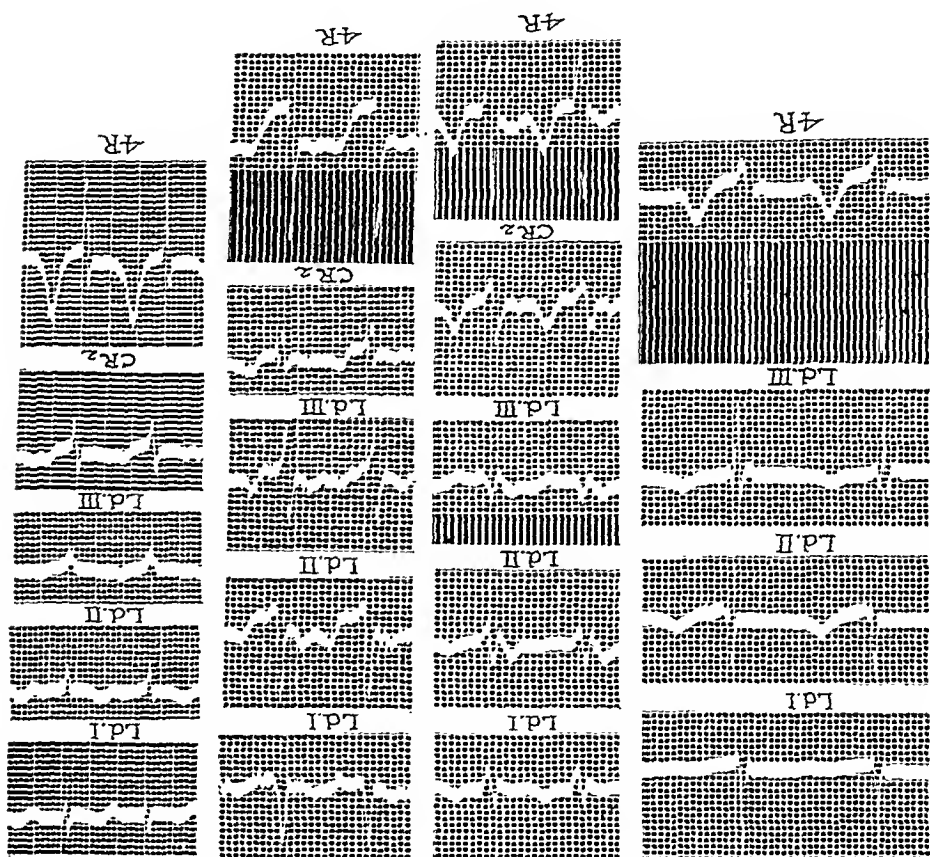


Fig. 2 (a-d).—Electrocardiograms of patients with pure lateral infarcts, with "typical" changes.

*Physical Findings.* Stupor, left hemiplegia, moderate venous distention, crepitant rales in the left base, enlarged heart, harsh systolic murmur at the apex, pulsus alternans, enlarged liver. White blood cell count 9450; increased sedimentation rate. She received digitalis during her hospital stay but the ECG was taken the day before admission when she had been digitalis-free for 1 month. On the 4th hospital day the patient became comatose and expired on the 5th hospital day.

*Pathologic Findings.* Recent infarct of the lateral and posterior wall 3 cm. from apex of left ventricle. Severe sclerosis and stenosis of left descending and left circumflex coronary arteries. Remote infarct of the posterior wall of the right ventricle. Cardiac hypertrophy and dilatation (625 gm.) (Fig. 1, e).

*ECG:* Left axis deviation. There was some elevation of the S-T segment in Leads II, III and 4R (Fig. 3, a).

Case 6. Male, age 55. *Clinical Diagnosis.* Coronary occlusion and multiple pulmonary emboli. Patient had attack of "acute indigestion" immediately following lunch on the day of admission. Severe substernal pain radiating into both arms followed by acute dyspnea and orthopnea.

*Physical Findings.* Rales in both bases of the lungs, enlargement of heart. Increased sedimentation rate; white blood cell count 13,050. The patient was digitalized. No digitalis had been given previous to the ECG.

*Pathologic Findings.* Recent infarct of anterior and apical portion of left ventricle. Remote infarct of lateral, posterior and basal portions of left ventricle. Recent thrombosis of descending branch of left coronary artery. Marked coronary arteriosclerosis. Focal acute fibrinous pericarditis. Hypertrophy and dilatation of heart (520 gm.) (Fig. 1, f).

*ECG:* Typical picture of posterior and basal infarction; auricular fibrillation (Fig. 3, b).

Case 7.—Female, age 85. *Clinical Diagnosis.* Recent coronary thrombosis; arteriosclerosis. Right hemiplegia 1 year before entrance. Increasing shortness of breath, orthopnea and cough for 1 year. One month previous to admission an attack of severe substernal pain lasting for about 1 week. This again occurred 1 day before admission and was followed by several attacks of vomiting.

*Physical Findings.* Mild dyspnea, rales in both lung bases, slight enlargement of heart to the left. Blood pressure 130/80. White blood cell count 9700 on admission; on 9th hospital day it was 13,000. Increased sedimentation rate. Patient was treated with nicotinic acid and digitalis without any response. She became comatose, had Cheyne-Stokes respiration and died on the 17th hospital day.

*Pathologic Findings.* Recent infarct of the lateral wall of the left ventricle with perforating aneurysm. Several small areas of fibrosis in the interventricular septum. Recent and remote occlusion of the left circumflex coronary artery for a distance of 5 cm. with a fresh thrombus in mid-portion. Sclerosis of coronary arteries. Pericardial adhesions. Hypertrophy and dilatation of heart (550 gm.) (Fig. 1, g).

*ECG:* Regular sinus rhythm (transient auricular fibrillation), and elevation of R-T<sub>1</sub>, and inversion of T<sub>4</sub> (Fig. 3, c).

Case 8.—Male, age 64. *Clinical Diagnosis.* Myocardial infarction, recent and remote; cardiac insufficiency; embolism of bifurcation of aorta; infarction of kidney. Two and a half weeks before admission, the patient developed an upper respiratory infection. Suddenly he became short of breath, did not respond to treatment and was hospitalized.

*Physical Findings.* Orthopnea, cyanosis, impaired resonance at bases of both lungs, decreased intensity of the breath sounds, many coarse and fine rales, enlarged heart, marked sclerosis of peripheral arteries. The liver was 4 cm. below the costal margin and was tender. Some ankle edema. White blood cell count 16,800. Shortly after admission the patient was digitalized. The diagnosis of saddle embolus was made and an embolectomy was done successfully. Patient died suddenly the following morning.

*Pathologic Findings.* Recent infarct of mid-lateral and posterior portion of left ventricle. Area of dense fibrosis in interventricular septum. Origin of left descending coronary artery occluded by recent thrombus. Right coronary artery occluded 2 cm. from origin by old occlusion. Coronary arteriosclerosis. Hypertrophy and dilatation of heart (510 gm.) (Fig. 1, h).

Case 9. Male, age 40. *Clinical Diagnosis.* Recent myocardial infarction; remote myocardial infarction; hypertensive cardiovascular disease. A diagnosis of coronary thrombosis with infarction of the posterior and basal portions of the heart was made 3 years before admission. Highest blood pressure was

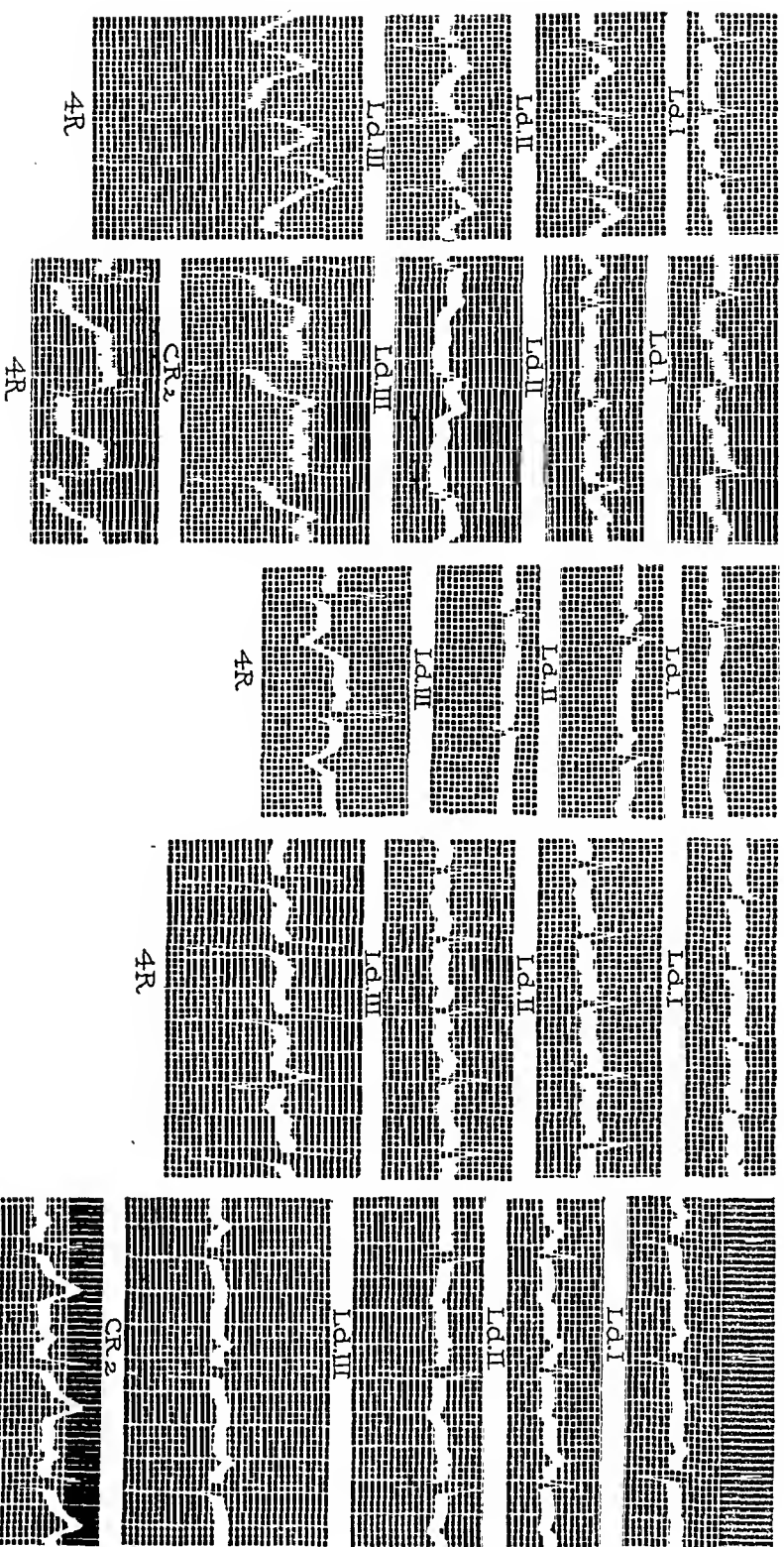


Fig. 3 (a-e).—Electrocardiograms of patients with pure lateral infarcts with atypical electrocardiograms.

160/110. The patient remained well and was able to work after his original coronary thrombosis until a few days before death when he had several anginal attacks. He died suddenly on Jan. 30, 1942.

*Pathologic Findings.* Recent and remote infarcts of lateral wall of the left ventricle. Recent thrombosis of left circumflex coronary artery and recent hemorrhage into wall of right coronary artery with organizing thrombosis. Hypertrophy and dilatation of right ventricle (Fig. 1, 2). There was no deviation of the RS-T segment (Fig. 3, e).

**Group III. Combined Infarcts.** In this group we include 3 cases of recent lateral infarction accompanied by other recent myocardial infarction. In 2 cases the interventricular septum was involved; in another case there was a coëxisting recent infarction of the apex of the left ventricle. Two records show evidence of a recent posterior and basal, and septal infarction. The other case showed left bundle branch block.

Case 10. Male, age 68. *Clinical Diagnosis.* Arteriosclerotic heart disease; coronary occlusion.

*Pathologic Findings.* Recent infarct of lateral and posterior wall of left ventricle, and posterior part of interventricular septum. Coronary arteriosclerosis marked. Left circumflex occluded by thickening of the wall. Recent hemorrhage and thrombosis of right coronary artery. Remote arteriosclerotic occlusion of left descending coronary artery with remote infarction of anterior wall of left ventricle. Hypertrophy and dilatation of heart (490 gm.). Fibrous pericarditis.

*ECG:* Evidence of posterior and basal infarction and right bundle branch block.

Case 11. Male, age 57. *Clinical Diagnosis.* Arteriosclerotic heart disease; coronary thrombosis; cardiac decompensation; right hydrothorax.

*Pathologic Findings.* Recent infarct of base and lateral wall of the left ventricle and anterior and apical portion of left ventricle. Severe coronary arteriosclerosis. Fresh thrombus in left circumflex coronary artery. Fibrosis of anterior and apical portion of interventricular septum. Hypertrophy and dilatation of heart (750 gm.).

*ECG:* Left bundle branch block.

Case 12. Male, age 50. *Clinical Diagnosis.* Arteriosclerotic heart disease; coronary occlusion; myocardial infarction, posterior and basal.

*Pathologic Findings.* Recent and remote infarction of lateral wall and apex of left ventricle, and basal portions of interventricular septum. Marked coronary arteriosclerosis and stenosis. Recent thrombosis of descending branch of the left coronary artery. Hemorrhage into wall. Marked fibrosis of endocardium. Acute fibrinous pericarditis. Hypertrophy and dilatation of heart (520 gm.).

*ECG:* Evidence of healed posterior and basal infarction and intraventricular block.

**Group IV. Remote Infarcts.** Four cases in this group had lateral infarcts extending into the posterior wall of the heart. Two extended into the anterior wall and only one was a pure remote lateral infarct.

Case 13. Male, age 69. *Clinical Diagnosis.* Hypertensive cardiovascular disease; generalized arteriosclerosis and dilatation of the aorta; severe heart failure; obesity; hypostatic bronchopneumonia.

*Pathologic Findings.* Remote healed infarct of lateral and basal portion of left ventricle. Marked arteriosclerosis and chronic fibrous pericarditis. Hypertrophy and dilatation of heart (540 gm.).

*ECG:* Taken before death showed auricular fibrillation. The QRS complexes were of low voltage and left axis deviation was present; a W complex was present in Lead II; the T wave was isoelectric in Leads I, II and III. There was a slight depression of the S-T segment in 4R.

*Case 14.* Male, age 58. *Clinical Diagnosis.* Myocardial infarction, posterior and septal; chronic bronchitis; emphysema and bronchiectasis.

*Pathologic Findings.* Area of scarring in central part of lateral wall of left ventricle; occlusion of left circumflex coronary artery by hemorrhage in an atherosclerotic plaque. Remote recanalized occlusion in right coronary artery. Severe arteriosclerosis with marked stenosis. Cardiac hypertrophy (470 gm.).

*ECG:* On admission this showed regular sinus rhythm, low amplitude of QRS; T<sub>2</sub> and T<sub>3</sub> sharply negative; slight elevation of the S-T segment in 4R with an inverted T. Another ECG taken 2 days later showed regular sinus rhythm, prolonged P-R interval, low voltage of QRS; T<sub>1</sub> isoelectric, T<sub>2</sub> and T<sub>3</sub> inverted. The T wave in 4R was sharply negative.

*Case 15.* Male, age 63. *Clinical Diagnosis.* Myocardial infarction, remote and recent; ventricular aneurysm; bronchopneumonia; arteriosclerotic heart disease.

*Pathologic Findings.* Remote infarction of anterior and lateral wall of the left ventricle with remote thrombosis of the descending branch of the left coronary artery. Marked arteriosclerosis. Hypertrophy and dilatation of heart (410 gm.).

*ECG:* Typical of remote anterior and apical infarction with interference dissociation.

*Case 16.* Female, age 64. *Clinical Diagnosis.* Hypertensive cardiovascular disease; coronary occlusion; decompensation; generalized arteriosclerosis.

*Pathologic Findings.* Remote infarct of apex and lateral wall of the left ventricle and apical portion of the interventricular septum. Marked stenosing arteriosclerosis. Recent thrombosis of the right coronary artery. Cardiac hypertrophy and dilatation (460 gm.).

*ECG:* The ECG of 3/15/41 had abnormal left axis deviation and the T wave in Lead I was diphasic. On 12/5/41, taken just before exitus, the ECG showed a prolonged P-R interval; low amplitude and notching of QRS in Leads I, II and 4R.

*Case 17.* Male, age 59. *Clinical Diagnosis.* Coronary occlusion; recent mid-thigh amputation.

*Pathologic Findings.* Remote infarction of lateral and posterior wall of left ventricle. Remote occlusion of left circumflex coronary artery; partial recanalization. Remote infarcts in the interventricular septum. Marked coronary arteriosclerosis and stenosis; mitral stenosis.

*ECG:* Showed auricular fibrillation and a deep Q in Leads II and III. Digitalis effect was marked. The patient had been on a maintenance dose of digitalis.

*Case 18.* Male, age 51. *Clinical Diagnosis.* Chronic glomerular nephritis; uremia; pericarditis.

*Pathologic Findings.* Remote infarct of lateral and basal wall of the left ventricle and interventricular septum. Marked coronary sclerosis and stenosis. Acute myocarditis of right auricle. Acute fibrinous pericarditis. Hypertrophy and dilatation of heart (600 gm.).

*ECG:* Left axis deviation and digitalis effect. (The patient was digitalized.)

*Case 19.* Male, age 66. *Clinical Diagnosis.* Arteriosclerotic heart disease. *Pathologic Findings.* Irregular areas of scarring of lateral and posterior wall of left ventricle. Marked arteriosclerosis and stenosis. Hypertrophy and dilatation of heart (560 gm.).

*ECG:* Auricular fibrillation and bigeminy with depression of S-T. This patient had digitalis intoxication.

**Remote Cases.** In 3 cases auricular fibrillation was present but in 1 case there was an associated mitral stenosis. The records taken showed no consistent pattern. Two records showed low amplitude of the QRS complex and in Case 13 there was a W complex in Lead II. Interference dissociation was present in 1 case. Digitalis intoxication dominated the ECG of 3 cases. Two cases showed changes of old posterior and basal infarction and 1 case of old anterior and apical infarction.

**Infarction of Lateral and Posterior Wall of the Right Ventricle.** CASE 20. Male, age 61. *Clinical Diagnosis.* Arteriosclerotic and hypertensive heart disease; diabetes mellitus.

*Pathologic Findings.* Recent and remote infarction of lateral and posterior wall of right ventricle. Recent and remote occlusive thrombosis of right coronary artery. Sclerosis of coronary arteries. Pericardial adhesions over area of infarction. mural thrombi in right auricle and right ventricle. Cardiac hypertrophy and dilatation (650 gm.).

*ECG:* Regular sinus rhythm, left axis deviation, slight depression of the S-T segment in Lead I and slight elevation of the S-T segment in Lead III. Lead 4R showed a small positive wave of the initial ventricular deflection. The record suggested a posterior and basal infarct. Weinberg and Katz<sup>12</sup> published the ECG of a patient with infarction of the lateral wall of the right ventricle showing a T<sub>p</sub> pattern.

**Confusion of Lateral Wall of the Left Ventricle.** CASE 21. Male, age 50. This case was a male who had received a close range bullet wound entering the fifth intercostal space, left mid-clavicular line and penetrating the pericardial sac in the mid-portion of the lateral wall of the left ventricle. There were irregular, slightly depressed and elevated areas 3.5 cm. in diameter, which showed acute focal interstitial myocarditis. Acute fibrous pericarditis was also present. The ECG showed changes found in pericarditis (epicarditis).

**Discussion.** The atypical ECG of cases of recent myocardial infarction have been studied by many authors.<sup>3,4,7-9,11,13</sup> The ECG may be altered by pericarditis (epicarditis); digitalization; combined infarcts, both old and recent; massive acute infarction; hypertension; coronary pulmonary; involvement of the right ventricle and intraventricular block. The ECG of recent infarction may be further distorted by myocardial hypertrophy and myocardial changes secondary to rheumatic heart disease. Robb and Robb<sup>10</sup> suggest that combinations of infarctions of the different muscle bundles may be responsible for atypical curves. Several writers<sup>4,5,8</sup> mention that involvement of the subendocardial layers of the myocardium may likewise be a factor. We have found in 4 cases of recent myocardial infarction the pattern described by Wood, Wolferth and Bellet,<sup>14</sup> including the high incidence of auricular fibrillation. Our findings emphasize the importance of the pattern of left lateral infarction. Further experience with extremity potentials<sup>5</sup> may increase the accuracy of diagnosis, especially of combined lesions.

**Summary and Conclusions.** 1. Nineteen cases of left lateral infarction were found in 106 cases of myocardial infarcts examined pathologically. Of these lateral infarcts, 9 were recent. In these recent cases the electrocardiograms of 4 (44.4%) showed the pattern described by Wood, Wolferth and Bellet.<sup>14</sup>

2. Atrial fibrillation or flutter was found in 5 of these recent cases (55.5%). This incidence may have been greater actually because all of the ECG showed atrial fibrillation or flutter to be temporary. In the 5 recent cases without ECG diagnostic of lateral infarction, the changes were diagnostic of posterior and basal infarction in 3 instances. The other 2 cases showed atrial fibrillation, and in 1 of these the ECG showed slight but not diagnostic depression of the S-T segment in Lead 4R.
4. Two cases associated with infarction of the posterior and basal region of the left ventricle presented ECG dominated by the T<sub>3</sub> pattern. One case associated with anterior and apical infarction had left bundle branch block.
5. Seven cases of remote lateral infarction showed no characteristic pattern.

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## ABDOMINAL AORTIC ANEURYSM

RUPTURE INTO THE JEJUNUM PRECEDED BY OCCULT BLOOD  
IN THE STOOL

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PROBABLY less than 25 cases of aneurysm of the abdominal aorta with rupture and perforation into the gastro-intestinal tract have been

reported to date.

In 1931 Riggs and Masey,<sup>6</sup> in a thorough review of the subject, were able to find 9 cases which terminated by rupture and perforation into the gastro-intestinal tract, 8 rupturing into the duodenum and 1 into the transverse colon. To this collection of cases they added another which ruptured into the duodenum. Since 1931 Scully,<sup>8</sup>



Neely,<sup>4</sup> Manson,<sup>2</sup> Roach,<sup>7</sup> Penas,<sup>5</sup> and Smith,<sup>9</sup> each reported a case with rupture into the duodenum, and Kampmeier<sup>1</sup> reported 1 with rupture into the stomach.

The rarity of this interesting phenomenon precludes any adequate statistical study of the symptomatology which may exist prior to fatal termination. However, the clinical findings of the case reported herein are such that they may add to those already recorded in the literature.

**Case Study.** The patient, a 76 year old white male, was admitted to the hospital on Dec. 2, 1942. He had enjoyed good health most of his life until 5 weeks prior to hospitalization, when epigastric pain and abdominal distention occurred with abrupt onset. At times the pain was cramping, knife-like and radiated across the upper abdomen. The pain was usually aggravated by eating solid foods. Nausea and anorexia were prominent features, and on one occasion he had vomited a large quantity of bitter tasting, greenish liquid material. Since the onset of symptoms he had lost 10 pounds in weight and complained of extreme weakness and dizzy spells. There was no history of tarry stools, dyspnea, or relief of pain by the use of antacids.

Physical examination at the time of admission to the hospital revealed an elderly, well-developed but poorly nourished individual who did not appear to be in acute distress. Temperature, pulse and respirations were normal. The head, ears, nose and throat were normal. The presence of bilateral immature cataracts precluded examination of the fundi. The chest was clear to percussion and auscultation. The heart was slightly enlarged to the left, the rate and rhythm were normal and no thrills or murmurs were noted. The systolic blood pressure was 120, diastolic 70. The abdomen was scaphoid in appearance. Moderate tenderness to palpation was present in the epigastrium. The liver was palpable 3 cm. below the right costal margin in the mid-clavicular line. No other masses and no pulsations were noted. Posteriorly, moderate tenderness to percussion was present over the region of each kidney. The genitalia, extremities, reflexes and rectal examination were either normal or non-contributory.

The hospital course was characterized by constant epigastric pain and anorexia, and intermittent nausea and abdominal distention. The temperature was elevated daily as high as 102° F. An admission blood count revealed 10.5 gm. of hemoglobin per 100 cc., 4,600,000 red blood corpuscles per c.mm. and 3200 white blood corpuscles per c.mm. Five urinalyses were normal except for slight traces of albumin. Examination of the gastric contents during the fasting state revealed a normal amount of free hydrochloric acid and no occult blood. No retention of food in the stomach was present on repeated aspiration. Roentgenologic examination of the upper gastro-intestinal tract revealed no demonstrable pathology in the esophagus or stomach. The duodenal cap filled to a normal contour. At 6 hours there was a slight trace of barium in the stomach, but the remainder appeared to be localized to the terminal ileum and cecum. There appeared to be a small calcified node anterior to the third lumbar vertebra. Roentgenologic examination of the colon by means of a barium enema revealed no defects, diverticula or polyps. Spasm was noted in the ascending and transverse colon. A 6 foot chest roentgenogram revealed the greatest transverse diameter of the thorax to be 27.6 cm., and the greatest transverse diameter of the heart to be 17.8 cm. The left ventricle was enlarged. Calcium plaques were present in the aorta. Scarring was present in the left apex and there were numerous small calcified foci in both lung fields. There appeared to be a slight pleural effusion on the left side and pneumonia in the base of the left lung. The guaiac test for occult blood in the stool was reported as follows: December 5, positive; December 7, slightly positive; December 11, positive; December 14, strongly positive; December 17, strongly positive.

On December 18 at 1:15 p.m. the patient was found to be semicomatose, the skin cold and clammy and the blood pressure unobtainable. A large quantity of dark red liquid feces was present in the bed. Despite immediate treatment for acute peripheral vascular collapse and acute blood loss the patient expired at 1:55 p.m.

**Autopsy Report.\*** The left pleural cavity contained 300 cc. of straw-colored fluid. On section, each lung presented a dark mottled appearance and considerable edema. The heart weighed 350 gm. The coronary arteries showed advanced atherosclerosis with some calcification. The thoracic aorta showed a number of atheromatous plaques measuring 1 to 2 cm. in diameter. A small aneurysm measuring 2 x 1.5 x 1.5 cm. was situated 18 cm. beyond the aortic valve. The abdominal aorta also showed many atheromatous plaques which measured up to 2 cm. in diameter.



Fig. 1.—Abdominal aorta showing atheromatous ulcer with "arteriosclerotic" aneurysm.

The intima of the abdominal aorta was covered by a soft thrombus which extended vertically from a level 3.5 cm. to a level 8.5 cm. above the bifurcation (Fig. 1). The thrombus measured 2 cm. transversely.

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at its upper portion and appeared to have an average thickness of about 1 cm. Its composition appeared to be old clotted blood, partially organized blood and atheromatous material. It appeared to rest upon an old ulcerated base which measured about 4 cm. vertically and up to 3 cm. transversely. The edges of the aortic intima adjacent to the ulcer were in part thinned and in part thickened. In the thick-



Fig. 2.—Specimen showing distal portion of duodenum (below and to left of ruler) and first portion of jejunum (above and to right of ruler) with perforation of jejunum (directly above ruler). Note portion of abdominal aorta (top) and iliac arteries (bottom) in background.

ened regions, there was considerable calcium deposit. The thrombus communicated with an aneurysm which was formed by dilatation of the ulcerated base of the aorta. The aneurysm measured 6 x 5 x 4 cm. and was filled with old and recently clotted blood. The wall of the aneurysm was found to be perforated at a level about 6.5 cm. above the aortic bifurcation.

Adjacent to the inferior pole of the aortic thrombus there was an irregular area of ulceration of the intima which measured about 1.2 cm. in average diameter and which was not covered by thrombus. Old blood clots and recently extravasated blood were present in the left retroperitoneal region. The stomach and first portions of the duodenum were normal. Much fluid and clotted blood was found in the remainder of the small intestine and in the large bowel including the rectum. The posterior wall of the jejunum showed evidence of erosion and, at a point 2 cm. from its junction with the duodenum, presented an irregular oval perforation about 1 cm. in average diameter. The long axis of this perforation was transversely disposed (Fig. 2). The mucosal edges of the perforation were soft and fairly smooth. A mass of dark blood, which had entered the lumen of the jejunum at the point of perforation, was connected with a mass of blood lying between the posterior wall of the jejunum and the aorta. The level of the perforation into the jejunum was found to be 2 cm. directly below the central point of the ruptured aneurysm. Direct communication between the jejunal perforation and the ruptured aortic aneurysm was demonstrated.



Fig. 3.—Section of aortic wall from near site of perforation showing destruction of inner two-thirds of media. Weigert elastic stain. ( $\times 95$ .)

Microscopic examination of the aneurysmal aortic wall immediately adjacent to the point of rupture showed marked thinning of the media with loss of muscle and elastic tissue (Van Gieson and Weigert elastic stains), and scattered and focal round cell infiltration of the adventitia (Fig. 3). A section of the jejunal wall bordering the area of perforation showed some loss of staining of the tissue with mild round cell infiltration of the various layers of the gut wall.

The liver weighed 1500 gm. and had a "nutmeg" appearance. The spleen measured 12 x 9 x 5 cm. and weighed 175 gm. It was slightly enlarged, soft in consistency and contained an increased amount of blood.

The pathologic diagnosis was ruptured arteriosclerotic aneurysm of the abdominal aorta with perforation into the first portion of the jejunum and fatal hemorrhage into the bowel; recent hemorrhage into the left retroperitoneal tissue; small arteriosclerotic aneurysm of the thoracic aorta.

**Comment.** The anatomic position of the third portion of the duodenum, as it crosses the vertebral column at the level of the third lumbar vertebra, brings it into close relationship with the aorta. Furthermore, this portion of the duodenum is relatively immobilized against the vertebral column and aorta by the pancreas, the mesocolon, the suspensory ligament of Treitz and other adjacent anatomic structures. This intimate relationship probably accounts for the predilection of aneurysms of the abdominal aorta, which arise in this region, to rupture into the third portion of the duodenum rather than into other portions of the small bowel. It also seems reasonable to assume that the pressure exerted by an aneurysm against this relatively immobilized portion of the small bowel accounts for some of the clinical findings and symptoms which are present. This is, in fact, substantiated by some of the cases reported in the literature.

Wills and Horton<sup>3</sup> report that 8.8% of all abdominal aneurysms are accompanied by gastro-intestinal symptoms. However, in their series of 80 cases, only 32.5% were aneurysms of the abdominal aorta. Kampmeier,<sup>1</sup> in a detailed study of 60 patients with aneurysm of the abdominal aorta, found pain to be present in 91.6% and "indigestion" in 20% of the cases. In general, abdominal pain, usually present in the epigastrium, and indigestion followed by anorexia and weight loss are the most frequent complaints of the patient. The duration of symptoms is usually less than 1 year, but has been recorded to last as long as 4 years. Washburn and Wilbur<sup>10</sup> report a case of abdominal aortic aneurysm causing obstruction of the duodenum which was subsequently relieved by posterior gastro-enterostomy. These authors also cite a similar case reported by Spizharney in 1907.

The roentgenologic findings, when present, are characterized by pressure defects on the structures adjacent to the aneurysm. Careful fluoroscopic examination of the upper gastro-intestinal tract is most important, for it may reveal and localize an extrinsic pulsating mass. Roentgenologic examination of the abdomen by means of lateral and anterior-posterior plates may demonstrate erosion of the vertebral bodies, displacement of organs or calcification in the aorta. Pyelography and barium enema examination are valuable for the same reasons—in the latter, if a pressure defect is present, it is usually in the left colon. In many instances, however, roentgenologic findings are absent or indeterminate and recourse to other clinical findings is necessary to arrive at the proper diagnosis.

The presence of occult or gross blood in the stool deserves special

attention. This finding, in the absence of any demonstrable cause and particularly when associated with gastro-intestinal symptoms, requires careful study and equally careful evaluation. Aneurysm of the abdominal aorta with pressure at some point on the gastro-intestinal tract should be included in the differential diagnosis of blood in the stool. In our review of the literature we found 3 cases in which blood was noted in the stool prior to rupture of the aneurysm with perforation into the bowel. In the case of Washburn and Wilbur,<sup>10</sup> occult blood was present in the stool on laboratory examination. The case reported herein showed the presence of occult blood in the stool for 13 days prior to rupture of the aneurysm with perforation into the jejunum.

**Summary.** A case of arteriosclerotic aneurysm of the abdominal aorta with rupture and perforation into the jejunum is reported. Aneurysm of the abdominal aorta with pressure on the small bowel is a rare cause of blood in the stool.

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## A TEST FOR VASCULAR TONE IN HUMANS AND ITS APPLICATION TO THE STUDY OF VASCULAR DISEASES WITH SPECIAL REFERENCE TO THE ETIOLOGY AND PREVENTION OF THROMBOPHLEBITIS

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For a long time those working with patients with vascular disease have felt the need for an objective method of measuring vascular tone.\* For the purpose of this investigation, "vascular tone" is defined as the ability of blood-vessels to constrict and dilate in response to cold and heat respectively, regardless of the mechanism involved. We are not using the term "vasomotor tone," which is frequently employed in discussing vasoconstriction and vasodilatation, because \*Tone has been defined by Evans<sup>1</sup> as the "resistance muscle offers to extension." Henderson<sup>2</sup> defines tone as "the peculiar vital form of muscular elasticity." Our definition of tone fits in with these in that ability of a vessel to resist vasodilatation with heat is analogous to ability of a muscle to resist stretching.

the latter phenomena, as we have observed them, are the end-result of an interplay of a variety of factors such as the degree of sympathetic tone, intrinsic smooth muscle tone of the blood-vessel wall, changes in blood volume, pressor substances and possibly other factors. We know, however, that the amount of sympathetic tone usually plays the predominant rôle in determining the state of vasoconstriction and vasodilatation.

Very little work has been done on the measurement of individual differences in vascular tone, because no clinical test was available. In patients, the diagnosis of vasospasm or abnormal vasoconstriction frequently is made solely on a clinical observation of coldness and cyanosis of the extremities in the presence of peripheral pulses. We have always regarded this diagnosis as inadequate, in that no measurement could be made of the degree of vasospasm or deviation from the normal, since the range of normal was unknown. A method has been developed by us, by which the degree of individual vascular tone can be determined by a simple clinical procedure, namely, the determination of the rate of fall in temperature of the extremities during a cool period and the rate of rise in temperature of the extremities during a period of application of moderate heat to the trunk in a constant temperature room at 20° C.

The clue to the test came from an observation made while performing routine reflex vasodilatation tests as described by Gibson and Landis,<sup>8</sup> which we had commonly used to determine the presence and degree of local organic arterial occlusion, depending on the height to which the temperature of the affected extremity rose in response to (reflex) heat to the unaffected extremities. It gave us no estimate of the peripheral vascular tone of the individual. By taking routine finger temperature determinations in addition to toe temperature readings and using only *moderate* heat, we found that we had a means of grading vascular tone in any individual. The importance of finger temperature readings lies in the fact that normally there is less tone in the vessels of the fingers than in the vessels of the toes, so that by observing the response to cold and to heat at these two sites, we obtain a gradient of vascular tone or ratio of vascular response in the feet to that in the hands in each individual. This gradient of vascular tone in the toes to that in the fingers was maintained throughout all of our studies in normal individuals. An additional point that is worth emphasizing is that only by using a stimulus of moderate intensity were we able to grade vascular tone. Had we applied more heat, as, for example, the warm water bath that was used in the original Gibson and Landis vasodilatation test, or had the electric pads been set at high heat, most normals would have vasodilated,\* and we could not have distinguished those who are moderately vasospastic. Vascular tone was determined by noting the rate at which the hands and feet cool, or vasoconstrict, in a cold room; and whether they become warm, or vasodilate with heat.

\* We realize that the term "to vasodilate," as a verb, is not recorded by Webster, though he has defined "vasodilating" as an adjective. However, the use of "to vasodilate" is common in clinical discussion and the term has been used in this paper because of its simplicity and clarity.

LOW VASCULAR TONE

		TABLE 1.—REFERENCE FOR DETERMINING VASCULAR TONE						
		LOW VASCULAR TONE					HIGH VASCULAR TONE	
		1	2	3	4	5	6	7
Group:	1							
	Remains HIGH (above 25° C.)	drops (below 25° C.)	drops (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)
Too temperature: During cool period								
	Remains HIGH (above 25° C.)	rises (above 30° C.)	rises (above 30° C.)	rises (above 30° C.)	rises (above 30° C.)	NO RISE From temperature during cool period	NO RISE From temperature during cool period	NO RISE From temperature during cool period
During heat period								
	Remains HIGH (above 25° C.)	rises (above 30° C.)	rises (above 30° C.)	rises (above 30° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)
Finger temperature: During cool period								
	Remains HIGH (above 25° C.)	rises (above 30° C.)	rises (above 30° C.)	rises (above 30° C.)	NO RISE From temperature during cool period	NO RISE From temperature during cool period	NO RISE From temperature during cool period	NO RISE From temperature during cool period
During heat period								
	Remains HIGH (above 25° C.)	rises (above 30° C.)	rises (above 30° C.)	rises (above 30° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)	LOW at start (below 25° C.)



applied to the trunk. With this information we made up a simple table into which individuals can be placed on the basis of vascular tone and from which the grade of vascular tone can be determined for any individual (see Table I). With this classification we were able to obtain the range in ability to vasoconstrict and vasodilate in normal individuals. Thus we have a basis of comparison for determining the presence or absence of abnormal vasoconstriction or vasodilatation in patients.

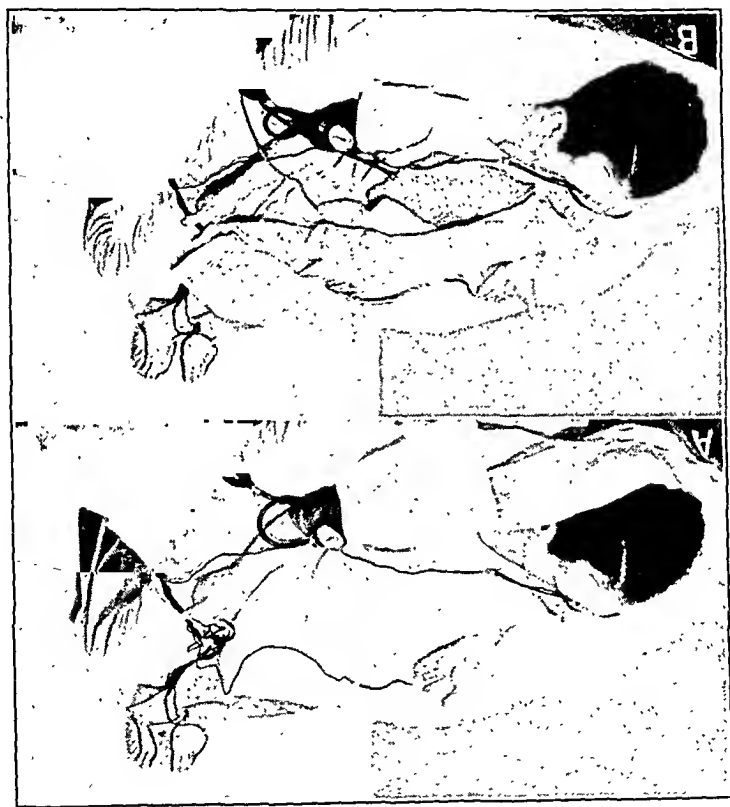


Fig. 1.—A, Test for vascular tone. Period of cooling. Temperature of room 20° C. Corner of blankets turned up to show position of electric pads. B, Period with reflex heat. Temperature of room 20° C.

**Method.** The subject is asked to omit the meal and any medication before the test. The test is described to the patient in general terms so that there will be no fear. The patient is asked to void before entering the test room to lessen nervous tension during the test. After entering the cold room, the temperature of which is maintained at 20° to 22° C. by a cooling unit, the subject undresses completely and puts on a light gown or sheet and lies in the supine position, his legs and arms being uncovered (see Fig. 1a). Thermocouples are attached to the first and third toes of each foot and the third fingers of each hand. The following routine measurements are taken 15 minutes after the patient lies down and every 10 minutes after the initial rise in skin temperature is observed following the application of heat: the temperature at each thermocouple; mouth temperature; blood pressure and pulse rate. When the toe and finger temperatures have dropped to as low a level as it appears they can fall (20° to

22° C.), requiring 30 to 60 minutes, 2 ordinary electric heating pads\* are applied to the trunk, covering it from the pubis to the neck. Two blankets are then used to cover the patient, excluding, however, the feet and ankles, arms and head (see Fig. 1b). The pads are turned on 5 minutes prior to being used so that they are warm when placed on the patient. The switch on the pads is set at medium heat. Readings are then taken as during the cold period, with the exception that time of onset and degree of sweating are recorded. Blood pressure and pulse rate determinations are not necessary in the routine grading of vascular tone, but have been taken by us to observe the changes taking place during the cool and warm periods in individuals with different degrees of vascular tone. If the subject has high-grade vascular tone (the vasospastic type), the finger temperature and the toe temperature will rise slowly or not at all in response to reflex heat. The average period with heat ranges from 60 to 150 minutes, individuals with high-grade tone requiring more time to vasodilate, if they can vasodilate at all with reflex heat. The temperature of the toes and fingers when vasodilatation is complete is between 30° and 34° C. When the toe and finger temperature responses to cold and heat have been obtained, the grade of vascular tone can be determined from Table 1. In some highly vasospastic subjects a posterior tibial nerve block with 3 cc. of 2% procaine confirms the diagnosis of high-grade vascular spasm. In testing normal individuals, one hardly requires a posterior tibial nerve block, since the manner in which the finger temperature responds to cold and heat will confirm the presence of vasospasm in the toes. Only the vasospastic individuals normally have cool hands at the start of the cool period; this is readily seen in Table 1. In the winter, subjects should be kept in a warm room for 30 minutes before starting the test. Typical tests done on a normal subject who vasodilates easily (low-grade tone) and on a normal subject who was vasospastic (high-grade tone) are seen in Figure 2.

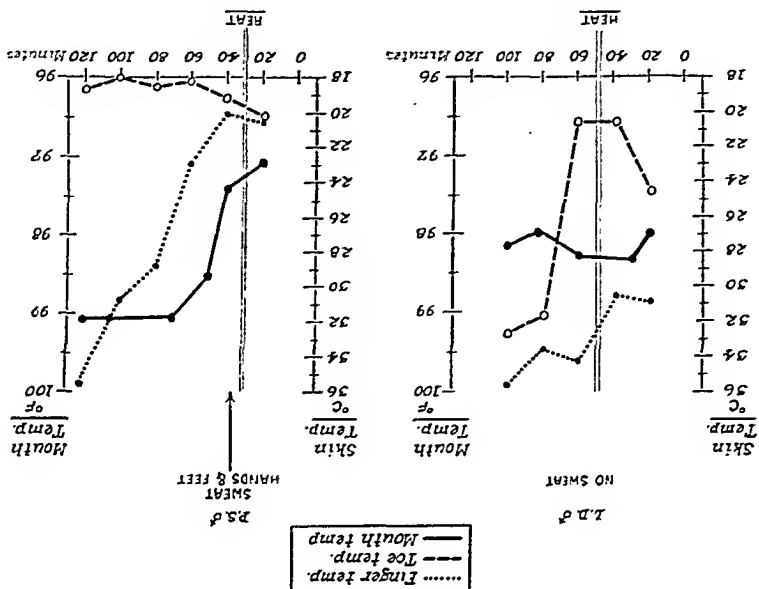


Fig. 2.—Responses during test of subject L.D. with low vascular tone (Group 2) and subject P.S. with high vascular tone (Group 6).

Results. The test has been performed on 172 individuals (including 119 patients and 53 normals as controls).

\* Westinghouse warming pad (cloth covered) 60 watts, 95-125 volts.

*Normal Individuals.* It was found early that normal subjects varied enormously in vasoconstrictor and vasodilator ability. The 53 normals tested had all grades of vascular tone with the exception of the most vasospastic grade (Table 2, Group 7). Normal individuals range all the way from those who are so dilated as the result of low vascular tone that they cool with difficulty in a cold environment, to those individuals who are always cold and respond slowly or not at all to reflex heat. The latter group of individuals is so vasoconstricted that they appear to behave no differently to reflex heat than patients with severe organic arterial occlusion in the legs.

TABLE 2.—PERIPHERAL VASCULAR TONE IN 53 NORMALS AND 119 PATIENTS

[illegible]

As the result of our observations we have been able to divide normal individuals into 2 main groups on the basis of their vascular tone. Those in Groups 1, 2 and 3 have low vascular tone as compared with those in Groups 5, 6 and 7, who have high vascular tone and are looked upon as being the vasospastic types. Group 4 is an intermediate group. Twenty-one of the 50 normals fell into Groups 1, 2 and 3. Twenty-eight of the 50 fell into Groups 5 and 6 (vasospastic groups). Too few normal subjects have been tested to attach any significance, statistically, to the number and percentage in each group. The response of male and female subjects during the vascular tone test is summarized in Table 3.

An individual will often prove to be vasospastic despite lack of a subjective history of cold hands and feet.

*Patients With Vascular Disease* (see Table 2). As might be expected, patients with known vasospastic diseases fell into Groups 5, 6 and 7. The 3 patients with Raynaud's disease fell into Groups 5 and 6. The 3 patients with acrocyanosis fell into Groups 5 and 6. Six patients with scleroderma fell into Groups 5, 6 and 7. Three patients with pernio fell into Group 6. Three patients referred by ophthalmologists with a diagnosis of retinal angiospasm all fell into Group 6. Of the 172 tests done, in only 3 patients was the response such that they could not be classified according to Table 1. In these patients

there was a disproportion in grade of vascular tone in the hands and feet, vascular tone being either much higher in the hands than in the feet, or very high in the feet and very low in the hands. One of these 3 patients had severe arthritic changes in the lower spine, which explained the local vasospasm in the legs. The other 2 patients had vasospasm in the hands, which was not true Raynaud's disease or acrocyanosis, and which we termed simple vasospasm of unknown etiology, possibly on the basis of a local mechanical or neurologic condition. It is conceivable that some patients may have low vascular tone in the legs and yet have vasospasm in the hands on the basis of a scalenus anticus syndrome, cervical rib, hemiplegia, pneumatic hammer disease, frost-bite or traumatic arterial spasm. When the response in either the hands or feet during the test is such that the patient cannot be placed anywhere in Table I, that individual has a pathologic grade of vascular tone such as purely local vasospasm or organic occlusion in the non-conforming extremities.

*Thrombophlebitis.* An unexpected finding was that the great majority of patients with thrombophlebitis were in the vasospastic groups. Sixteen of 17 patients tested had a high grade of vascular tone, falling into Groups 5 and 6. The 1 patient in the low vascular tone group had much milder symptoms of thrombophlebitis, and when seen a year later had recovered spontaneously. All of these tests were done in the warmer months. We then obtained from our files the records of the cases diagnosed thrombophlebitis seen in the year prior to the start of this investigation. Eighteen of the 20 patients seen had a history or evidence of cold feet or cold feet and hands. In addition, 2 patients who had been labeled by us clinically as being vasospastic, have since been operated upon and have developed thrombophlebitis.

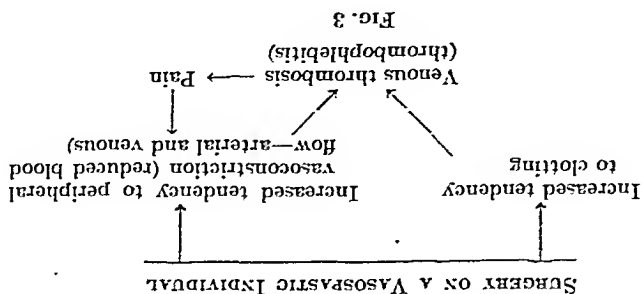


FIG. 3

We know that a certain percentage of normal individuals, the vasospastic normals, when vasokonstricted, cannot vasodilate for hours, even with electric pads, during which period blood flow is greatly reduced in the extremities. Furthermore, the veins also participate in the peripheral vasokonstriction. When a vasospastic individual is subjected to the vasokonstrictor, stimuli arising from abdominal and pelvic surgical procedures, which include anxiety and pain, plus the shortening of blood coagulation time, which occurs for several days following surgery, we have an ideal set-up for thrombosis to develop in the veins (see Fig. 3). In addition, once thrombophlebitis is

initiated, pain may cause further reflex vasoconstriction and further venous thrombosis. The knowledge that it is the individual with high vascular tone who is most susceptible to thrombophlebitis may answer the question that Evans<sup>8</sup> asks in a recent paper on thrombophlebitis: "Why do these epidemics of pulmonary embolism come in winter time?" The answer well may be that we are all more vasoconstricted in the winter, and the vasospastic individuals even more so. One of the reasons that phlebitis occurs almost always in the legs may be that it is in the lower extremities that vascular tone is greatest. Veal and Hussey<sup>17</sup> found that operative manipulations within the abdominal cavity cause an immediate rise in venous pressure in the saphenous vein without affecting the pressure in the antecubital vein. Davis, Gilman and Freedberg<sup>4</sup> found marked increases in venous pressure in the veins of the foot during abdominal operations.

It is possible that measures directed to prevent vasoconstriction in the legs may be of importance in reducing the incidence of postoperative thrombophlebitis and pulmonary embolism. These measures would include prevention of chilling such as by insufficient blankets or permitting the patient to be uncovered or in a draft. Prevention or rapid relief of shock to prevent peripheral vasoconstriction would be indicated. The recent introduction of leg exercises has reduced the incidence of pulmonary embolism considerably (Krebs,<sup>11</sup> Evans<sup>9</sup>). This type of exercise, by increasing blood flow and producing hyperemia, aids in maintaining vessels in a state of vasodilatation. Smith and Allen<sup>16</sup> found a reduced venous flow in the extremities postoperatively. Walters<sup>18</sup> has advised the use of thyroid to increase blood flow in the extremities postoperatively. Leriche<sup>13</sup> and also Ochsner and DeBakey<sup>14</sup> have popularized paravertebral ganglia block with procaine for acute thrombophlebitis. Their rationale for this is that the venous thrombus causes reflex arterial and venospasm, which plays a primary role in producing the full-blown picture of thrombophlebitis. However, knowledge of the fact that the vasospastic individual is the one most likely to be thrown into reflex vasospasm, and that he happens also to be the one most susceptible to thrombophlebitis, suggests that the vasospasm plays a role not only after the development of the venous thrombus, but in bringing it about. By looking upon thrombophlebitis as a disease in which the vasospastic state or preliminary abnormal vasoconstriction, venous and arterial, plays an important rôle, our concept of it becomes clearer, and prevention placed upon a more rational basis. The importance of prevention of thrombophlebitis is demonstrated by Gibson's<sup>7</sup> statistical analysis that of every 100 postoperative deaths, 8 are due to pulmonary embolism.

*Thromboangiitis Obliterans.* Nine of 32 patients with thromboangiitis obliterans were in the groups with low vascular tone, 1, 2 and 3. Prior to the development of this test for vascular tone, one could only have an impression that patients with this disease had or had not vasospasm. By measuring finger temperature responses to cold and heat, we can tell whether these patients are types with high or low vascular tone, even though they have severe organic arterial occlusion in both

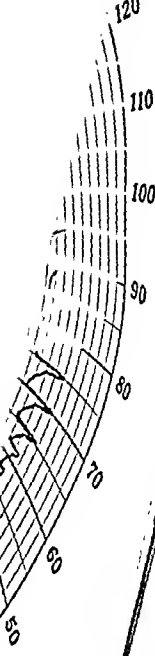
NAIDE: A TEST FOR VASCULAR TONE IN HUMANS

legs, or if both legs have been amputated. The 5 female patients with thromboangitis obliterans were all vasospastic. The effect of emotions on vascular responses was often observed during the test. Tension produced in a patient, on being told that a posterior tibial nerve block was to be done, often caused a drop of 2° to 6° C. The drop in temperature was always greater in the upper extremities. Conversation concerning subjects which worried the patient would cause a drop of several degrees in skin temperature. Vasospastic individuals than among those with low-grade vascular tone. In response to emotional stimuli was more prominent among vaso-

TABLE 3.—PERIPHERAL VASCULAR TONE IN 50 NORMALS AND 121 PATIENTS

Normals:		Patients:	
Low tone (Groups 1-3)	High tone (Groups 4-7)	Low tone (Groups 1-3)	High tone (Groups 4-7)
11	9	22	5
2	42	5	46
8	16	1	5
3	16		
Male			
Female			

*Location of Vasoconstriction and Vasodilatation.* A question which arises in our measurement of constriction and dilatation is that of its location along the vascular tree. Are the vessels that are constricting and dilating large arteries, small arteries, arterioles or capillaries? By combining oscillometric readings with the vascular tone test, a clue is obtained in some patients. Oscillometric recordings are taken at the wrist and ankle. In vasospastic individuals (normals and patients) oscillometric readings or pulsations were usually small during the cool period. With vasodilatation the pulsations, as recorded by the other hand, with increased markedly in some subjects, but increased only slightly or not at all in others. The interpretation of this can be increase in blood flow must be accomplished through dilatation of vessels that are smaller than can be recorded by the oscillometer. On the other hand, with increased pulsation during vasodilatation, the increase in blood flow must have been accomplished through dilatation of arteries of the radial and ulnar, or dorsalis pedis and posterior tibial, which are known to record on the oscillometer. In an individual whose vasoconstriction and dilatation occurs in vessels smaller than the caliber of the wrist and ankle arteries, the oscillometric readings will be moderate large and of the same size during vasospastic restriction and vasodilatation (Fig. 4). In this type of vasospastic circulation will have no increase in zero oscillation and an adequate collateral skin temperature rises to normal during vasodilatation. This is an objective demonstration that vessels of a lesser caliber than the major



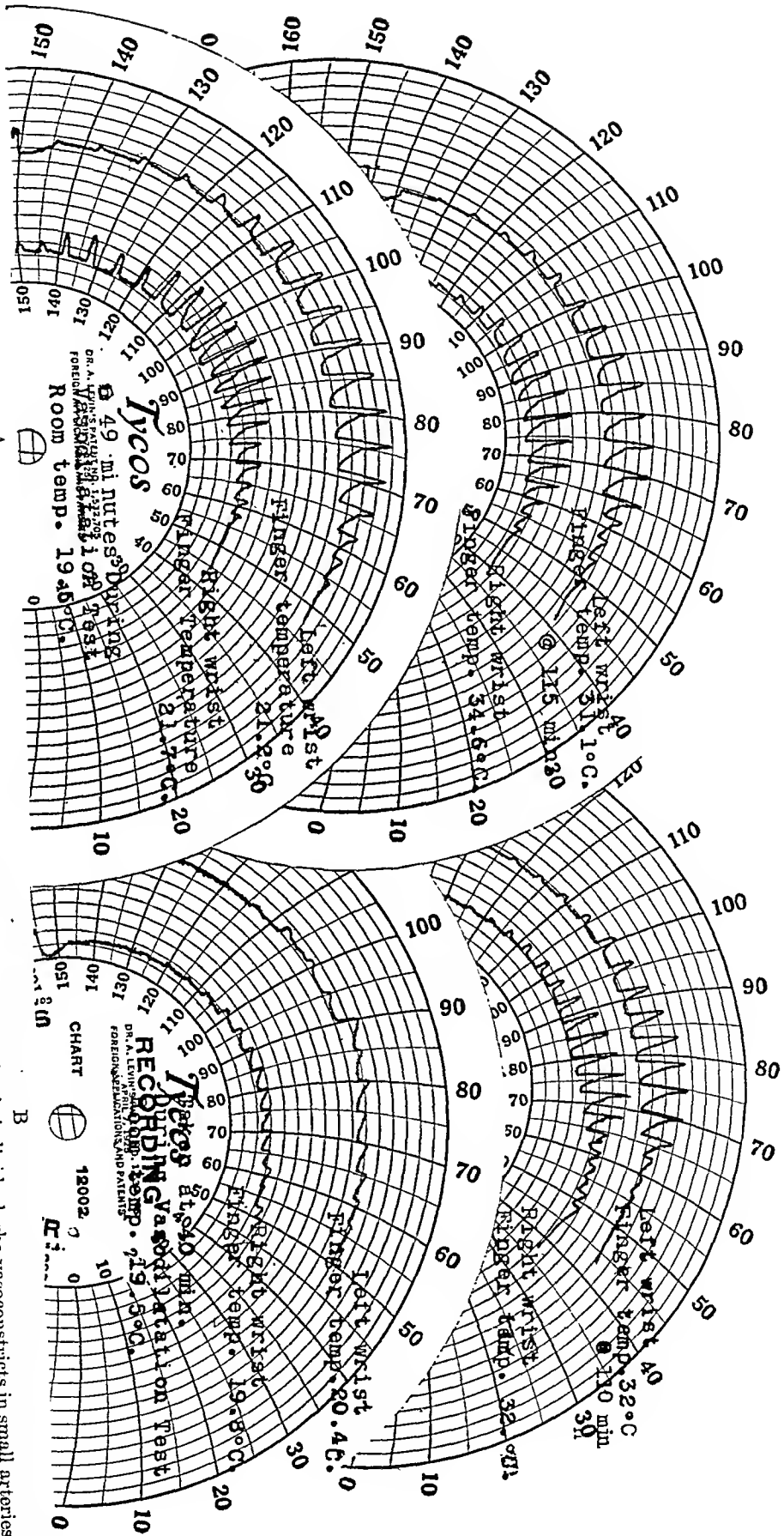


Fig. 4.—A, High vascular tone (Group 6). Oscillographic readings unchanged during vasoconstriction and vasodilatation in individual who vasoconstricts in small arteries or arterioles. B, High vascular tone (Group 6). Oscillographic readings increased during vasodilatation in individual who vasoconstricts in arteries of large caliber.

arteries, even though dilated, are not recorded by the oscillogrometer. It must be emphasized that oscillographic recordings alone cannot evaluate individual vascular tone. With the vascular tone test, however, oscillographic recordings taken during the cool and warm periods will, in some subjects, point to the site of vasoconstriction and vasodilatation. The importance of these observations lies in the demonstration that vasoconstriction and vasodilatation normally may take place in arteries of different caliber in different individuals.

*Heat Elimination.* As part of the study, the onset and degree of sweating and changes in mouth temperature were recorded routinely. As the result of difficulty in eliminating heat through vasodilatation, vasoconstrictive individuals often sweat earlier, and to a greater degree, than those with low-grade vascular tone (see Fig. 2). Mouth temperature usually rises to a higher level with heat in the vasoconstrictive subjects. Vasoconstrictive individuals frequently, but not always, give a history of sweating more and having higher mouth temperatures than those subjects who eliminate heat readily through vasodilatation. The vasoconstrictive individual, as a rule, is more comfortable in warm weather. The individual with low vascular tone cannot tolerate hot weather as easily as cold.

*Blood Pressure Responses During the Test.* Individuals with high vascular tone, both normals and patients, had a rise in blood pressure during the cool period more frequently than did those with low vascular tone. The stimulus produced by exposure to cold for a prolonged period on the blood pressure is not of the same order as that produced during the cold pressor test where the stimulus is sharp and short, a hand being placed in ice-water for 2 minutes. The significance of the cold pressor test, at present, is uncertain except that individuals are classified as being either hyporeactors or hyperreactors, according to the blood pressure rise during the test.

Seven of 16 patients with hypertension whose vascular tone was determined by our method fell into the low tone groups (see Table 2). As yet, too few hypertensive subjects have been tested in this manner to draw any conclusions.

*Pulse Rate During the Test.* During the test the pulse rate rose or fell just as frequently in vasoconstrictive subjects as in those with low vascular tone. There appears to be no relationship between a change in cardiac rate and the ability to vasoconstrict and vasodilate.

Because the metabolism of the individual has an effect on blood flow in the extremities, it was felt that the influence of this factor on vascular tone, as we grade it, should be investigated. Table 4 summarizes the basal metabolic rate in 31 subjects. Although the metabolic rate may influence blood flow somewhat, its effect on vascular tone, as we determined it individually, appears to be slight.

The 3 patients with high basal metabolic rates (+17, +34 and +43) were markedly vasoconstrictive, 2 of them being in Group 7. In such patients the increase in sympathetic tone producing vasoconstriction may be greater than the effect of the increased metabolism, which



would otherwise tend to produce vasodilatation. Twenty-nine of the 31 basal metabolic rates were done on subjects with high vascular tone. Seventeen of these had normal basal metabolic rates. In 3 of the 7 patients with high vascular tone and a low basal metabolic rate, the figure was only —11. Although vascular tone, *per se*, does not appear to be greatly influenced by the basal metabolic rate, it is possible that a low basal metabolic rate in a vasospastic individual will further reduce blood flow and be an additional factor in predisposing an individual to postoperative thrombophlebitis. The use of thyroid post-operatively to prevent thrombophlebitis, as recommended by Walters, certainly appears rational in such a situation.

TABLE 4.—BASAL METABOLIC RATE AND VASCULAR TONE IN 6 NORMALS AND

25 PATIENTS			
Low B.M.R. below —10	1 patient	14 patients	3 patients
	Normal B.M.R. —10 to +10	1 normal	None
High B.M.R. above +10	7 patients	3 normals	3 patients
	2 normals	3 normals	3 patients

*Vascular Tone and Blood Volume.* As warm weather approaches, peripheral vasodilatation increases in order that body temperature will remain normal. Bazett<sup>1</sup> has found that the blood volume increases in the spring to take care of both internal organ needs and also that of continued increased peripheral blood flow. In the fall, however, with gradual peripheral vasoconstriction as the result of lower environmental temperature, the blood volume decreases. Since this is true, it may be that vasospastic individuals have lower blood volumes than individuals with low vascular tone.

*Reactions of the Pupil During the Vascular Tone Test.* Early in this investigation we observed that individuals who fall into the vasospastic group usually have strikingly large pupils, particularly the group with thrombophlebitis. As a result of this observation we made routine measurements of the diameter of the pupil during vasoconstriction and reflex vasodilatation. The light surface overhead, at which the patient's vision was directed during measurement of the pupils, was kept constant at 2.69 milliambers throughout the test. The diameter of the pupil was measured with the pupillometer described by Bourbon.<sup>2</sup> Pupils measuring 5 mm. or more were considered large under the light conditions of this investigation. During vasodilatation the pupils became smaller in most of the patients and normals—markedly so in some, less so in others. Langworthy<sup>3</sup> has pointed out that the pupil dilates when the vessels of the iris constrict and becomes smaller when the vessels fill with blood. We know that constriction of the blood-vessels in the iris is brought about through increased sympathetic tone, and for this reason we consider the larger pupil, in individuals with more than average tendency to peripheral vasoconstriction, additional clinical evidence of increased sympathetic tone as the cause of increased peripheral tone. A few vasospastic individuals had small pupils. In them, increased vascular tone did

not appear to be of sympathetic origin. This was true in a few of the normal individuals, as well as in 2 of the 3 patients with Raynaud's disease whom we have tested. This fits in with the recent concept of Lewis and others that the fault in Raynaud's disease lies primarily in the blood-vessel wall and not in increased sympathetic tone.

**Discussion.** This test on normal individuals and patients has given us a measuring rod for vascular tone, which permits us to investigate problems where it plays a rôle.

It is probable that the behavior of a vasospastic individual with peripheral organic arterial disease differs from that of an individual with low vascular tone with the same degree of organic arterial disease. Clark<sup>3</sup> has observed the disappearance of arterioles in the rabbit's ear with "lessening of blood flow—persistent maintenance of a completely contracted condition." On the other hand, he found that increased blood flow may lead to the formation of numerous arterio-venous anastomoses in stable vascular networks. This suggests that the rate of development and patency of collateral vessels in patients with organic arterial disease may be related to their peripheral vascular tone.

The finding that many normal persons are so vasoconstricted that they may be easily confused with patients with peripheral vascular disease makes it quite conceivable that occasionally a vasospastic individual will be needlessly sympathetomized. This could occur should he complain of symptoms in the extremities on a basis other than vascular disease and where the examiner could find no pulses in the feet and no rise in toe temperature with an ordinary vasodilatation test which measured only the changes in the affected extremities. However, this type can be differentiated from patients with organic arterial disease by observation of the fact that the hands also participate in the vasoconstriction and can disclose the diagnosis of high-grade vascular tone. Furthermore, nerve block with procaine will cause vasodilatation in the vasospastic individual who fails to respond to reflex heat, but not in the patient with organic arterial occlusion. It may be asked why nerve block in a vasospastic individual will not suffice for determination of vascular tone. A glance at Table 1 discloses that only the individuals in Groups 6 and 7 are proper subjects for this test, as it is only in these 2 groups that the extremities fail to respond to reflex heat. None of the individuals in Groups 1, 2, 3, 4 and 5 could be differentiated by nerve block alone, yet they represent tremendous individual variations in ability to vasoconstrict and vasodilate.

The importance of the part that vasospasm plays in the etiology of thrombophlebitis has been disclosed by the test. It is interesting that thrombophlebitis is common in the Scandinavian countries, where vasoconstriction would be assumed to be the result of a cold climate. Various problems are yet to be worked out relating the degree of vascular tone to other pathologic conditions. For example, what relationship is there, if any, between the degree of vascular tone and the ease with which shock develops? Does the individual with low

vascular tone have a more or a less efficient mechanism to withstand shock? What is the significance of the fact that some hypertensive patients fall into the low vascular tone group, whereas others are vasospastic? What relationship is there, if any, between the information obtained by this test and that obtained by the cold pressor test? Individuals who proved to be hyperreactors with the cold pressor test did not show signs of vasospasm during the vascular tone test. The complexity of the problem is illustrated by the fact that during the cold pressor test (placing a hand in ice-water for 1 minute while observing blood pressure responses in the opposite arm) we found little or no drop in skin temperature of the toes, even in a hyperreactor. Yet telling such a patient that he will be stuck with a needle for a posterior tibial nerve block causes a drop of 2° to 6° C. in skin temperature, which reflects a considerable reduction in blood flow in the hand. Furthermore, leaving such a patient in a cool room for 30 to 60 minutes results in a 10° C. drop in toe and finger temperatures, demonstrating that different stimuli have different effects on blood vessels. The vascular tone test offers us a measure for evaluating the role that individual vascular tone or ability to vasoconstrict and vasodilate may play in a variety of diseases. We are continuing the typing of normal individuals and patients with various vascular diseases with the hope that the separation of individuals into vascular types may help us in the study of the etiology of certain vascular diseases and the varying response to therapy in different patients with the same disease.

Summary. A clinical method for the determination of "vascular tone" in humans is described.

Results of vascular tone typing, carried out on 172 individuals, have revealed the wide range of vascular tone in normal persons and patients and its significant role in the etiology and course of certain vascular diseases and in the selection of appropriate therapy.

The importance of vasospasm in initiating thrombophlebitis or phlebotrombosis has been disclosed by the test.

This test can indicate whether vasoconstriction and vasodilatation in different individuals is taking place in large or small vessels.

The relation of vascular tone to basal metabolic rate, blood volume, blood pressure, cardiac rate, heat elimination, emotional stimuli and size of the pupil have been studied and discussed.

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# ACUTE THROMBOCYTOPENIC PURPURA IN INFECTIOUS MONONUCLEOSIS

## REPORT OF A CASE

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In the past 10 or 15 years many additions have been made to our knowledge of infectious mononucleosis, and the protean manifestations of this somewhat mysterious disease entity are becoming more and more familiar. The occurrence of various hemorrhagic phenomena is now recognized as not being extremely unusual, although in earlier descriptions of mononucleosis it was stated categorically that purpuric and petechial eruptions did not appear.<sup>1</sup> Epistaxis, however, has always been a common incident in the course of the disease and as early as 1895 observers recorded that nosebleeds might be the precursor of glandular fever.<sup>2</sup> Tidy and Daniel<sup>3</sup> reported a school epidemic of 24 cases of which 8 had one or more epistaxes at various stages of the illness. Similarly hematuria has been a fairly common manifestation of infectious mononucleosis and in a series of 270 cases collected in 1921 as many as 6% were found to show urinary bleeding.<sup>4</sup> The hematuria, while it may be severe, is not usually associated with casts or functional disturbance and rarely if ever is the precursor of acute nephritis. Rectal bleeding has also been reported but is seldom a prominent feature.

At present the development of hemorrhagic skin eruptions is recognized as an episode of moderate frequency and merely constitutes one of a great variety of rashes which have been described in this disease. Petechial and purpuric hemorrhages may occur anywhere in the skin or mucous membranes, larger ecchymoses may be seen, and in some cases a positive tourniquet test may be found—in all these instances the blood platelets being normal in number and no other indications of a hemorrhagic diathesis being present.<sup>5</sup>

The occurrence of a true acute thrombocytopenic purpura in conjunction with infectious mononucleosis is, however, exceedingly rare, and in fact only a very few unimpeachable cases may be found in the literature. Williams<sup>6</sup> in 1931 described his own experience with glandular fever in which he developed a purpuric rash with a positive tourniquet test and prolonged bleeding time, but no platelet count was done. Alnout<sup>7</sup> in 1936 reported 3 cases in which severe thrombocytopenic purpura was accompanied by lymphocytosis and some varying

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degree of lymph node enlargement. In only 1 of these was the heterophil agglutination test performed, however, and it proved to be negative. Downey and McKimlay<sup>3</sup> in presenting a group of 9 cases described 2 in which purpuric phenomena appeared, but again no platelet counts were made and no gross hemorrhages occurred. On the other hand, Cottrell's group of 12 cases contained 1 in which a platelet count of 77,600 was reported but no hemorrhagic manifestations were described,<sup>2</sup> and in another instance a platelet count of 45,000 was found in the absence of any evidence of bleeding.<sup>1</sup>

Perhaps the most authentic case of true purpura and mononucleosis is that recorded recently by Magner and Brooks<sup>5</sup> in which hematuria, bleeding gums, purpuric eruption, prolonged bleeding time, and estimated reduction in platelets were combined with lymphocytosis, palpable spleen, moderate enlargement of the inguinal nodes, and a Paul-Bunnell test of 1:800, the entire condition clearing up in 6 to 8 weeks. Inasmuch as this clinical picture is one of extreme rarity, the addition of a similar instance to the list seems warranted and the present case is herewith reported:

**Case Report.** M. S., age 30, 2d Lt., Chem. Warfare Dept., U. S. Army, Jewish, was admitted to the 2d Gen. Hosp. on Oct. 15, 1942, complaining of weakness, fever, and sweats of 3 weeks duration, and red spots appearing on tongue and lips in the preceding 3 to 4 days.

**Family History.** Several members of his family had hay fever. His mother had rheumatism. There was no history of tuberculosis, diabetes, cancer, or diseases of the blood.

**Personal History.** He had been a pharmacist's assistant in civilian life and had been in the army 22 months. He thought he had lost about 4 to 5 pounds in the past 6 months. He smoked 8 to 10 cigarettes daily and used alcohol rarely.

**Past History.** His general health had been excellent. He had had no serious illness or operations, no allergic reactions, no tendency to bleed or bruise easily, and no disease involving the lymph nodes. His cardiorespiratory system had been essentially negative. There were no gastro-intestinal symptoms save occasional heartburn and what he called sour, nervous stomach. He had never been jaundiced. He had had no symptoms referable to the genito-urinary tract and venereal disease was denied. It is of interest to note admission to the hospital.

**Present Illness.** He felt in his usual state of good health until about mid-July when after 2 or 3 days of being unusually tired he noticed that his urine showed a pinkish discoloration. He did not seek medical advice and after a short period of rest the condition cleared up. Thereafter he felt well until in early September he experienced gradually increasing fatigue and weakness, without localizing symptoms. About the first of October he began to feel feverish at times and to have night sweats. Thinking he had a gripply cold he stayed in quarters for 3 or 4 days, but his temperature reached 102° F. and on Oct. 10, 1942, he was admitted to the 180th Station Hospital where a diagnosis of acute bronchitis was made and he was treated with ephedrine nose drops and gripe capsules containing aspirin, phenacetin and caffeine. At no time did he receive any quinine, sedormid or sulfonamide drugs.

On the 3rd hospital day he complained of several sore spots in his mouth and was found to have numerous hemorrhagic blebs on his tongue and buccal mucosae and a few petechiae scattered over his body. He continued to have fever reaching 101° F. daily and was weak and lethargic. More ecchymotic spots appeared on his lips, tongue and oral mucosa, he had nosebleeds, and

TABLE 1.—LABORATORY FINDINGS

Date (1942)	Hgb. (%)	R.B.C. (mill. per cmm.)	W.B.C. (thous. per cmm.)	Seg- mented (%)	Lym. (%)	Mono, (%)	Eos. (%)	Baso, (%)	Platelets (thous. per cmm.)	Sediment rate (mm./1 hr.)	Paul- Bunnell test	Urine	Stool
Oct. 15	91	4.8	11.8	35	54	10	0	1	66	50	..	Dark red; grossly bloody; albumin 4+	Guaiac 4+
10	..	4.1	8.8	20	77	2	1	..	..	..	..		
21	..	3.0	7.0	38	58	3	..	..	..	..	..		
24	80	4.7	8.3	41	52	3	1	1	76	..	1:512	Grossly bloody	Guaiac negative
Nov. 2	102	4.0	10.4	58	34	3	2	1	116	33	..		
13	92	4.0	..	..	..	..	..	..	..	..	..		
20	..	..	13.3	61	32	3	1	1	148	25	..		
30	..	..	9.8	50	46	10	2	2	174	27	..		
Dec. 5	..	..	9.1	51	35	1	..	..	202	..	1:64		
12	..	..	8.5	60	38	1	1	..	..	..	..		

*Blood Smears.*—Previous to November 20 showed numerous large lymphocytes and mononuclears with many atypical forms. No lymphoblasts seen. Platelets were extremely scarce in the early smears.

*Additional Laboratory Work.*—October 15: Blood culture sterile. Bleeding time, 1 minute. Clotting time, 6 minutes. October 22: Total serum protein, 7.06 mg. per 100 cc.; serum albumin, 4.13 mg. per 100 cc.; serum globulin, 2.93 mg. per 100 cc.; hetero index, 0; serum phosphorus, 3.56 mg. per 100 cc.; serum phosphatase, 6.72 mg. per 100 cc.

*Sternal Puncture.*—October 21: Smear of the sternal marrow shows numerous atypical mononuclear cells similar to those seen in the peripheral blood. No abnormality of the various bone marrow elements is detected.

began to pass grossly bloody urine. Two days later he was transferred to this hospital for further study.

On careful questioning into the possibility of exposure to chemicals it was discovered that there had been no contact with any of the benzene series or with any gases other than those used routinely and in small amounts for training purposes several months previously. During the summer there had been occasional exposure to the fumes of tetrachlorethane, a non-corrosive decontaminant which is known to be a powerful hepatotoxin but has not been found to produce any significant change in the hematopoietic system.

*Physical Examination.* On admission the patient's temperature was 101° F. His color was sallow but he was not obviously anemic, nor was there evidence of marked weight loss. He displayed alternately nervous excitement and extreme exhaustion. His eyes were negative externally and no hemorrhages were made out in the optic fundi. There was dried blood in the nares and the nasopharynx showed evidence of marked congestion. The tip of the tongue, buccal mucosae, and lips presented numerous ecchymotic spots, some measuring nearly 1 cm. in diameter. The gums were normal. The pharynx was dusky red but there was no exudate.

The right tonsillar node was about 1.5 cm. in diameter. There were numerous small, shotty post-cervical nodes on either side. One or 2 small nodes were palpable in the axillae, and the right epitrochlear node was felt. The lungs were clear. The heart was normal. Blood pressure 110/70. The abdomen was held tense but there was no spasm, and no masses or tenderness were made out. The liver and spleen were not palpable. The inguinal nodes were small and not impressive. The genitalia were negative. Scattered groups of petechiae were seen on the lower legs and in the right flank. The deep reflexes were somewhat overactive and equal. There was no edema. *Laboratory Work.* (See Table 1.) A Roentgen ray of the chest was negative.

*Course.* For the first 5 days the temperature showed a daily rise to nearly 101° F., then subsided. During this time the patient's condition remained essentially unaltered, and gross hematuria persisted. Hemorrhagic spots remained in the mouth and a few fresh petechiae were noted on legs and trunk but there were no further nosebleeds. The patient complained mainly of complete exhaustion and drenching sweats were frequent, but at no time was there any severe prostration or evidence of collapse. Marked postnasal congestion continued to be a troublesome symptom and considerable exudate was obtained on nasal irrigation. After the 5th day the temperature remained normal and improvement was steady thereafter. By November 2 there was no evidence of bleeding of any kind and the purpuric eruption had practically disappeared. The lymph nodes showed no further enlargement and the spleen was at no time palpable. Convalescence during November and December was marred only by a mild acute enterocolitis and later by an upper respiratory infection with abundant nasal discharge. Occasional herpetic lesions appeared in the oral mucosa but these were transient, and there was no recurrence of any of the purpuric phenomena.

The patient was discharged symptom-free on Jan. 11, 1943.

*Discussion.* Despite the absence of significant lymphadenopathy and palpable spleen it is felt that the characteristic lymphocytosis with numerous large lymphocytes and atypical mononuclear forms, combined with a heterophil agglutination of 1:512, establishes the diagnosis of infectious mononucleosis beyond a doubt. The explanation of the associated thrombocytopenia on the other hand is a difficult problem, because in the absence of anemia, leukopenia and sternal marrow changes one can scarcely postulate a depression or dysfunction of the bone marrow. Presumably a combination of vascular

damage as a result of the acute infection, together with loss of circulating platelets at the purpuric sites, is responsible for the widespread hemorrhagic phenomena.<sup>10</sup> The low platelet count in the circulating blood may be an expression of a transient toxic effect exerted peripherally rather than centrally.

It is well known that severe infectious mononucleosis may in its early stages be indistinguishable from acute lymphatic leukemia, and the presence of purpura has long been considered an important diagnostic point in favor of the latter. In the light of the case at hand, however, it is obvious that purpuric and hemorrhagic phenomena with or without thrombocytopenia cannot constitute a valid distinguishing feature between these two conditions. The finding of a well-marked anemia, on the other hand, is exceedingly rare in mononucleosis, and if such a condition appears without obvious explanation, one may well suspect the presence of a leukemic process.

**Summary.** 1. A case is reported of infectious mononucleosis with associated acute thrombocytopenic purpura.

2. Hemorrhagic skin eruptions, epistaxes, and hematuria are infrequent findings in infectious mononucleosis, but do occur even in the absence of thrombocytopenia.

3. Purpuric phenomena even with marked platelet reduction do not necessarily constitute a significant diagnostic point in favor of leukemia.

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## THE "HEAT RESISTANCE" OF ERYTHROCYTES

## A SPECIFIC TEST FOR THE RECOGNITION OF MARCHIAFAVA'S ANEMIA\*

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MARCHIAFAVA'S anemia is a relatively rare clinical entity. Its symptomatology consists chiefly of nocturnal hemoglobinuria, constant hemosiderinuria, pseudomacrocytic anemia with a strong tendency to regeneration of the bone marrow, increased reticulocyte count in the peripheral blood, and leukopenia. In making the differential diagnosis, other forms of hemolytic anemia and hemoglobinuria must be con-

\* Translated by Morris Kramer, B.A., M.D.



sidered. As the latter symptom at times retreats almost completely into the background, a lack of nocturnal excretion of hemoglobin does not automatically exclude the disease.

The osmotic resistance test of the erythrocytes allows the demonstration of the presence of spherocytes, and has therefore taken on particular importance in the diagnosis of Marchiafava's anemia or hemolytic anemia. For the diagnosis of Marchiafava's anemia, however, this test is useless, as the fragility of the red cells is not increased. This disease, as the investigations of Ham<sup>1-3</sup> and Heggin and Maier<sup>4,5</sup> have shown, is due to the action of a hemolysin whose ambocceptor is always present in the erythrocytes. In the presence of complement hemolysis takes place. The degree of hemolysis increases as the temperature of the blood rises, and as the pH of the blood falls.

While it is technically rather awkward to test the acid-base balance of the blood, it is on the contrary quite easy to determine the resistance of the red blood cells to increased temperature. The test consists of putting a test tube containing about 5 cc. of blood obtained by means of a dry-air sterilized syringe into the incubator for 6 to 24 hours at a temperature of 37° C. The degree of hemolysis can then be observed with the naked eye.

A quantitative determination of the degree of hemolysis, *i. e.*, the hemoglobin content of the serum, is from a practical standpoint unnecessary. After making the above test in numerous cases of anemia we have, nevertheless, determined the hemolysis according to the method of Wu Hsien.<sup>6</sup>

The results are as follows:

No.	Name	Age (yrs.)	Diagnosis	Serum hemoglobin values at 37° C.	
1	B. H.	28	Marchiafava's anemia	After 6 hours	After 24 hours
2	S. F.	44	Marchiafava's anemia	675	1357
3	G. R.	64	Acquired (?) hemolytic anemia	6	23
4	G. A.	39	Constitutional form of hemolytic anemia	35	180
5	G. L.	27	Constitutional form of hemolytic anemia (jaundice)	33	51
6	H. A.	35	Probably acquired form of hemolytic anemia (jaundice)	14	13
7	K. A.	36	Paroxysmal hemoglobinuria after exposure to cold	11	
8	P. E.	15	Toxic hemolysis (pyrogallol acid)	96	142
50 normal cases					
7 cases of secondary anemia					
Hemolysis not increased					
12 cases of pernicious anemia					
Hemolysis not increased					

In Case 7 (paroxysmal hemoglobinuria brought about by exposure to cold), an increase in hemolysis could be observed if immediately after venous puncture the blood was first placed in a refrigerator for 30 minutes, and then brought into the incubator (Donath-Landsteiner).

In Case 8 (toxic hemolysis through external application of pyrogallie acid), distinct hemolysis was already evident on taking the blood out of the vein. This, however, did not increase on being further warmed in the incubator.

In pernicious anemia, if the blood remains in the incubator for 48 hours at 37° C., one may at times observe a slight hemolysis. However, this never attains a value over 150 mg. per ml. It is evident from the above table that the blood in Marchiafava's anemia tends to hemolyse on application of warmth. It should also be mentioned that the hemolysis increases greatly when the blood is shaken from time to time while in the incubator. From the incidental degree of hemolysis *in vitro*, however, it is impossible to draw conclusions regarding the possible phase or stage of the disease. Summary. The concept of the "heat resistance" of erythrocytes is herewith presented. If hemolysis of clotted blood *in vitro* is evident to the naked eye after 6 hours at 37° C., it appears to be pathognomonic, specific evidence of the presence of Marchiafava's anemia.

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#### HOMOLOGOUS SERUM JAUNDICE\*

#### A REVIEW OF THE LITERATURE AND REPORT OF A CASE

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A POSSIBLE hazard involved in the clinical use of biologic preparations containing homologous blood products is suggested by the recent reports of cases of jaundice following their parenteral administration. The importance of this problem is well illustrated by the 1942 outbreak of hepatocellular jaundice in the United States Army. This parenteral injection of certain lots of yellow fever vaccine, and although the exact etiology has not yet been determined, it seems to have been related to the human serum component of that vaccine. \* This investigation was aided in part by the Commission on Measles and Mumps, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon-General, A. U. S.

However, in large bodies of men living in crowded or otherwise abnormal conditions, it is difficult to exclude the possibility of transmission by some other means, and for this reason, the study of an isolated case in civil life may be of value in the further analysis of this problem. We have been led, therefore, to report the case of a physician who developed severe and prolonged hepatocellular jaundice 11 weeks after the intravenous injection of a pooled mumps convalescent plasma.

**Review of Literature.** The important data on the major outbreaks of jaundice that have been reported as occurring in relation to the administration of biologic products containing homologous blood factors are tabulated below. One notes that the disease has been associated with the use of glycerinated humanized smallpox lymph,<sup>18</sup> yellow fever vaccine,<sup>3,8,9,13</sup> measles convalescent serum,<sup>21</sup> pooled mumps convalescent serum,<sup>21</sup> whole blood transfusions,<sup>21</sup> pooled and dried reconstituted human adult serum,<sup>23</sup> and pappataci fever vaccine.<sup>27</sup> Analogous cases have been reported after immunizing procedures involving the use of homologous sera in horses.<sup>4,29</sup>

**Etymology.** Since the other components of the biologic products have seemed to be eliminated as factors in the production of the disease process, the injected blood, plasma or serum naturally has been suspected of carrying the ieterogenic agent. Indeed, that human blood and some of its derivatives may contain a transmissible ieterogenic agent has been demonstrated by Oliphant and his associates,<sup>25</sup> who were able to transmit the disease to human volunteers by the subcutaneous injection of serum obtained from patients in the preicteric or icteric stages of postyellow fever inoculation jaundice. Cameron<sup>2</sup> and Voegtli<sup>31</sup> also have succeeded in producing jaundice in volunteers by subcutaneous or intramuscular injection of serum or blood from patients with infective hepatitis. Findlay and Martin,<sup>11</sup> by introducing the nasal washings of patients with postyellow fever inoculation jaundice into the nasopharynx of volunteers and observing the subsequent occurrence of jaundice in a significant number, demonstrated that the agent may be present in the nasopharynx. All attempts to transmit the disease to animals have thus far been unsuccessful.<sup>2,25</sup>

Extensive studies of these ieterogenic blood products, however, have failed to reveal the exact nature of the causative agent; and yet certain properties, including its transmissibility in serum, its lack of visibility under the microscope, its passage through bacteria-retaining filters, its persistence in serum-chick embryo media and its resistance to various procedures which ordinarily kill vegetative bacteria have led to the belief that it may be a virus. At present, this concept cannot be denied or confirmed.

The clinical and pathologic pictures of this disease and those of infective hepatitis\* are so similar that some feel they are identical and due to the same causative agent. Others, however, believe that different etiologic agents are concerned. The various opinions are summarized in a recent *Lancet* editorial<sup>7</sup> which suggests three possibilities. \* The term infective hepatitis, as used here, is synonymous with catarrhal jaundice, infectious hepatic jaundice and epidemic jaundice or hepatitis.

TABLE 1.—STATISTICAL DATA ON SOME REPORTED GROUPS OF CASES OF HOMOLOGOUS SERUM JAUNDICE\*

Source of report (date)	Location	Suspected source of heterogenic agent	Number of subjects injected	Number of cases of jaundice	Incidence of jaundice (%)	Number of deaths	Incubation period (weeks)	Duration of jaundice (weeks)
Lurman, <sup>1</sup> 1885	Brechen	Glycerinated humanized lymph (smallpox vaccine)	1,289	191	14.8	.....	4 to 28	
Findlay <i>et al.</i> , <sup>2</sup> 1939	Varied	Yellow fever vaccine	2,200	48	2.2	.....	8 to 32	
Circ. Letter 95, Surg- Gen. Office, <sup>3</sup>	U. S. Army	Yellow fever vaccine	.....	28,585	.....	62	10 to 13 (average)	4 to 8 (average)
Fox, Manso, Penna and Para, <sup>13</sup> 1942	Brazil	Yellow fever vaccine	.....	.....	.....	.....	12 to 20 (average)	5 to 24
		Lot No. 489	9,604	736	7.7	.....	(average)	
		Lot No. 494	9,587	150	1.5	25	2 to 78	
		Other lots	87,987	93	0.1	(from Nos. 489 and 494)	(extremes)	
Sergiey, <sup>21</sup> 1940	.....	Pappatuci fever vaccine	360±	109	30.0	.....	9 to 21	
Mem. M. O. Min. of Health, <sup>21</sup> 1943	England	Pooled measles convales- cent serum (IX-60)	109	37	37.6	8	2 to 16	
		Measles adult serum (No. 488)	.....	11	.....	1	11 to 23	
		Pooled mumps convales- cent serum	266	86	32.3			
	A.R.C. Harvard Unit	Mumps convalescent serum	.....	48	.....	.....	5 to 19	
Morgan and William- son, <sup>22</sup> 1943	England	Reconstituted dried serum; pooled plasma	56	9	16.1	.....	7 to 16	
Beeson, <sup>1</sup> 1943	Grady Hospital, Ga.	Blood or plasma transfu- sions	.....	7	.....	.....	4 to 29	
Oliphant <i>et al.</i> , <sup>23</sup> 1943	Virgin Islands	Yellow fever vaccine	1,039	153	14.7	0	10 to 19	1
		Experimental inoculation volunteers	189	30	15.8	0	4 to 19	(average) 1 to 7
		Yellow fever vaccine						
Cameron, <sup>2</sup> 1943	Experimental inoculation volunteers	Blood or serum from pa- tients with infective hep- atitis	7	6	86.0	0	4 to 24	

\* The statistics for certain groups are incomplete as the table includes only those appearing in the literature to date.

ties, namely, that the two diseases are caused by: (1) the same virus, (2) different strains of the same virus, or (3) different agents producing the same clinical picture.

In support of the proposition that the infective hepatitis virus is at least one of the causes of serum jaundice is the recent report of Cameron<sup>2</sup> concerning the development of jaundice in volunteers inoculated with blood or serum from patients with infective hepatitis. His cases represented serum jaundice in which the icterogenic agent was almost certainly that of infective hepatitis, and, furthermore, the interval between the inoculation and the onset of the disease was as long as 6 months, suggesting that the difference in the length of the incubation period of the two diseases does not necessarily indicate different etiologic agents. Against the hypothesis of the infective hepatitis virus as the only cause of serum jaundice is the apparent absence of contact infection. This was observed as a striking feature of the United States Army outbreak related to yellow fever vaccine.<sup>3</sup>

As in other diseases, individual susceptibility or predisposition appears to play an important rôle. Some investigators<sup>2,9,13</sup> are of the opinion that manifestations of the disease may occur only after some other factor has rendered the individual susceptible. Cameron<sup>2</sup> feels that the onset may be delayed until general resistance is lowered, for example, by conditions (fatigue, malnutrition, exposure, etc.) such as occur among the men of an army in the field. Thus his volunteers did not develop jaundice until they had spent some time on active field service.

*Pathologic Anatomy.* Dible and his associates<sup>6</sup> have studied the hepatic pathologic changes in 56 cases of non-fatal acute hepatitis, the specimens being obtained by aspiration biopsy. The group was composed of 14 cases of epidemic hepatitis, 5 cases of jaundice following the injection of mumps convalescent serum (American Red Cross Harvard Unit group), 2 cases of jaundice following the transfusion of serum and 35 cases of arsenotherapy jaundice. A hepatic inflammation of varying intensity and distribution was common to all and, regardless of the etiology, one type of case could not be differentiated from another by histologic criteria. The essential changes consisted of hepatic cell necrosis and autolysis, associated with leukocytic and histiocytic infiltration. The centers of the lobules showed the first of these changes to the most marked degree, and the portal tracts the greatest cellular infiltration. In cases mild from the beginning, or in which the lesion was retrogressing, the perportal cell accumulations predominated, in contradistinction to the more severe cases in which hepatic cell degeneration was more pronounced and the leukocytic and histiocytic infiltration more widespread. Some cases exhibited nodular hyperplasia and cirrhotic changes. As a rule, diffuse hepatitis was found to heal completely and rapidly, though in cases running a longer course, residual fibrosis persisted in the portal zones even after apparent clinical cure. The mechanism of the production of icterus was thought to be due to the disruption of the liver-cell columns and their intercellular bile canaliculi, the functional inadequacy of the

damaged cells and the plugging of the canaliculi with bile thrombi. There was no evidence of bile stasis in the interlobular branches of the bile ducts, further confirming the intralobular nature of any obstruction to the outflow of bile that existed.

The main hepatic changes in the fatal cases of jaundice following yellow fever vaccination in the United States Army were those of acute or subacute yellow or red atrophy.<sup>3</sup> The investigators of the cases that occurred in Brazil<sup>11</sup> reported similar observations, occasionally finding, however, the changes of infective biliary cirrhosis. Other abnormalities in the United States Army cases included marked edema and intense inflammation of the gastro-intestinal tract, usually most pronounced in the cecum, bile nephrosis, acute or subacute splenic tumor and hemorrhages in various locations.

*Clinical Features.*<sup>3,9,13,21,25</sup> The incubation period or interval between the injection of the suspected product and the development of jaundice is regarded as one of the most important distinguishing features of this disease and is usually quite long, varying from 2 to 32 weeks, the average being 8 to 12 weeks. The onset may be asymptomatic or with a "grippe-like" syndrome similar to that of infective hepatitis. During the preicteric period, polymorphic rashes, urticaria and joint pains are common and have been emphasized by some as especially characteristic of this type of hepatitis. Abdominal pain may be present and occasionally may simulate closely that of acute appendicitis. Anorexia, nausea and vomiting are frequent and either diarrhea or constipation may be present. The temperature is often normal or only slightly elevated. Jaundice gradually appears in most cases and the course thereafter is similar to that of infective hepatitis. Worthy of mention is the fact that some authorities are convinced that the disease may occur without icterus. Severe cases, such as occurred in one outbreak in England due to measles serum, may exhibit irritability, restlessness, intractability, delirium, screaming, extensor plantar reflexes, hematemesis and bloody stools. Pruritus may be severe and may occur in the preicteric period.

The physical observations are also similar to those of infective hepatitis. Dehydration is often marked. The liver may be enlarged and tender, but in the less severe cases is often not palpable. The spleen is often palpable during some stage of the illness.

*Laboratory Observations.*<sup>3,13,21,25</sup> If the disease is severe or of long duration, anemia may occur. The leukocyte count is usually normal, though leukopenia is not uncommon. The differential count occasionally reveals an increase in monocytes. Greenblatt and Kaplan<sup>14</sup> recently reported the presence of an unusually high percentage of "target cells," a variant of red blood corpuscles, in the circulating blood of 22 patients with postyellow fever inoculation jaundice. They are characterized by a small central mass of hemoglobin surrounded by a clear area, which in turn is circumscribed by an additional ring of hemoglobin; such cells are said to be more resistant than normal to hemolysis by hypotonic saline solution. They, however, are also found in other conditions and thus are not specific for serum jaundice.

The urine usually contains bilirubin and urobilinogen, and bile salts may be present. The stools may be acholic for variable periods. The serum bilirubin and icterus index are of course elevated. Liver function tests show varying degrees of disturbance of hepatic function. The histamine test may be positive in the preicteric period.<sup>2</sup>

**Prognosis.** Recovery in 4 to 8 weeks was the rule in the United States Army cases<sup>3</sup> and, in most instances, was complete. In a few, persistent liver damage apparently resulted. The mortality was approximately 0.2%. The highest mortality reported was in one of the civilian groups in England ascribed to measles serum, 8 deaths occurring among 37 cases.<sup>21</sup>

**Prophylaxis.** The only prophylactic measure thus far recommended has been the rejection of potential blood donors who give a history of recent non-surgical jaundice.<sup>1,10</sup> It would also seem advisable to report all cases of serum jaundice to an appropriate authority so that the cause may be investigated and further therapeutic use of the suspected product prevented.

**Case Report.** A male physician, 43 years of age, developed, in August 1942, an attack of jaundice that persisted in an intense form for more than 3 months. The initial diagnosis was infective hepatitis, and not until the severity of the process necessitated a complete reevaluation of the case was the significance of the preceding history fully appreciated.

**History.** The past medical history contained one important item, a 3 weeks illness in 1933 characterized by fever as high as 102° F., marked jaundice and some hepatic enlargement. It was regarded as a typical case of catarrhal jaundice ("infective hepatitis"). He was then in good health until June 1942, when he contracted mumps from one of his children. During this illness he developed considerable anorexia, nausea, epigastric discomfort and tenderness, suggesting the possibility of a complicating pancreatitis, and 200 ml. of pooled mumps human convalescent plasma were therefore administered intravenously. He recovered promptly and was again in good health until mid-August 1942, when after 2 to 3 days of vague epigastric discomfort scleral icterus was first noted (approximately 11 weeks after the injection of the plasma). The intensity of the jaundice progressively increased, and on the 40th day of his illness, because of anorexia, dehydration, weight loss and general discomfort, he was admitted to the University Hospital for more vigorous treatment and study.

The jaundice reached its maximum on the 43rd day. Anorexia was marked, pruritus severe, mental depression constant, and he had lost 30 pounds in weight. Symptomatic and objective improvement then began and, except for a temporary relapse between the 52nd and 63rd days, was progressive. Visible jaundice persisted, however, until between the 90th and 117th days, during which interval he was not under observation. His pruritus also disappeared during that period and he regained his normal weight level. Subsequently he was permitted gradually to resume his normal activities and he has since been in good health.

**Physical Observations.** When first seen, early in the disease, physical examination was entirely negative except for the obvious jaundice. The liver, however, soon became palpable and slightly tender and during the mid-stage of the disease, its edge was palpable as far down as the level of the umbilicus (6 cm. below the costal margin). Though more firm than normal, it then became gradually smaller but was still vaguely palpable on the 117th day. Between the 32nd and 34th days the spleen was just palpable, but was never felt thereafter. The skin was exceedingly dry and the site of numerous excoriations until the late phase of the illness; it then gradually assumed a

more normal character and the excoriations healed rapidly. Bradycardia was a constant feature, and the temperature was never elevated. *Special Examinations and Studies.* Blood studies showed nothing unusual except a gradual drop in the hemoglobin level to 10.1 gm. (52nd day). Leukocyte and differential counts were not abnormal. The urine was deeply colored from the onset, and the first examination revealed urobilin, bilirubin and bile salts. The latter disappeared (Hay's and Oliver's tests) about the 50th day, though the serum bilirubin 2 days later was 22 mg. per 100 ml. of blood. Bilirubinuria was not demonstrable after the 85th day (serum bilirubin, 6.4 mg. per 100 ml.). Routine urinalyses were otherwise consistently negative. During the mid-stage of the disease, a urea clearance test showed no significant abnormality of renal function. The blood urea nitrogen values were always normal.

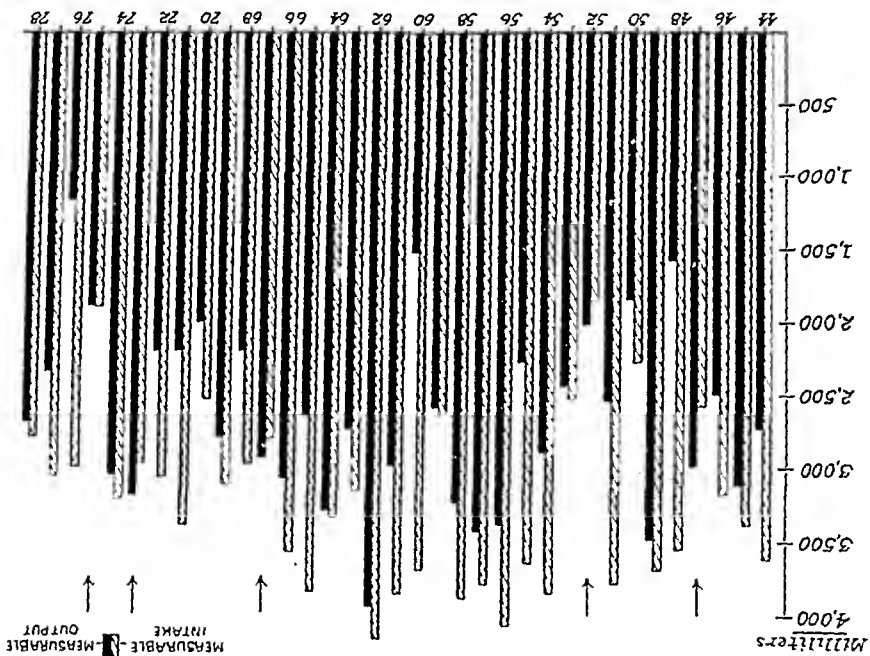


CHART 1.—Measurable intake and output of fluid during 44th to 78th day of the disease. Previously an oliguria had been present. Arrows indicate days on which output equaled or exceeded intake; abscissae, days of disease.

The stools were light in color or truly acholic from the onset of the jaundice until the 46th day, after which the bile content steadily increased. During the early stage of the disease, quantitative measurement of the intake and output of fluids was not possible, but the patient stated that a very definite oliguria occurred. By the time the jaundice had reached its height (43rd day), however, a pronounced diuretic tendency was noted (Chart 1), and this persisted until the time of the patient's discharge from the hospital (83rd day). The serum bilirubin level, as determined by the method of Malloy and Evelyn,<sup>12</sup> rose progressively until the 43rd day, when a value of 41.6 mg. per 100 ml. was obtained (Chart 2). The level then gradually dropped to 22 mg. (52nd day). At that time a temporary relapse occurred, with an aggravation of all the symptoms, and the serum bilirubin persisted at about 22 mg. until the 64th day, after which it again gradually fell. On the 117th day, the level was still slightly elevated (1.9 mg.). When again checked 3 months later, its concentration was normal.



Studies of the blood cholesterol and its ester fraction (Chart 2) yielded results compatible with those usually found in hepatocellular jaundice. The concentration of the total cholesterol was low normal during the early stage of the disease, while that of its ester fraction was below normal. Both gradually increased with the fall in the serum bilirubin level and general clinical improvement, the value of the total actually rising to high normal toward the termination of the disease. The last ester fraction value shown on the chart, which is low, is unexplained, but may have been due to a technical error since the concentration was normal when checked a few weeks later.

### Liver Function Studies

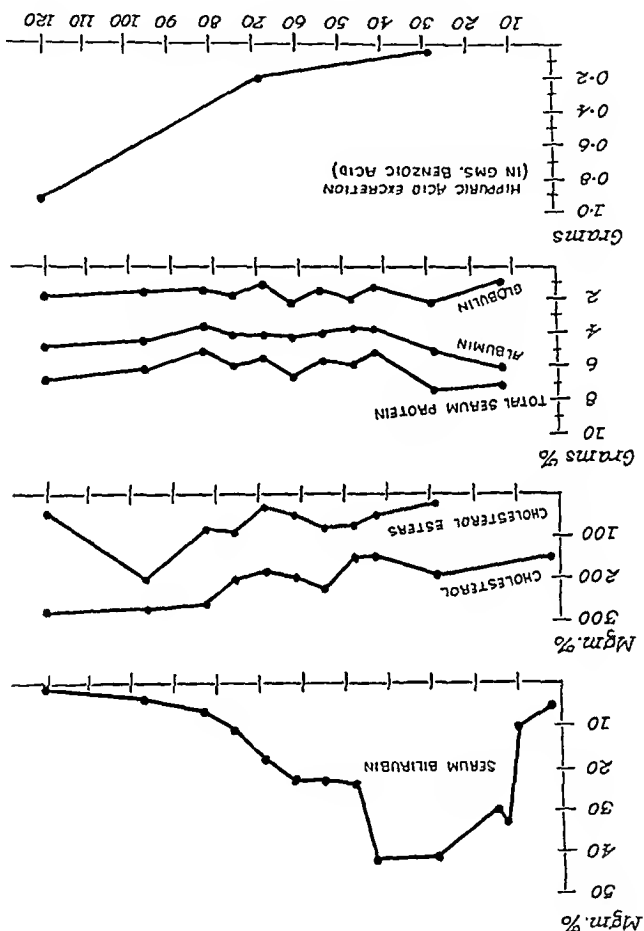


CHART 2.—Data on the serum bilirubin, cholesterol and protein concentration in the blood serum and on the results of the hippuric acid excretion and protein tests. Abscissae indicate days of disease.

Serum protein studies (Chart 2) were frequently made and the total protein values were not unusual. Likewise, the serum albumin values, except for a surprisingly high concentration (6.1 gm. per 100 ml.) on the 12th day of jaundice when the initial determination was made, were not unusual, the level gradually falling to a minimum of 3.9 gm. per 100 ml. during the mid-stage of the disease. The globulin level, however, in spite of the duration and severity of the illness, was usually low or low normal, the average of 12 determinations being 1.7 gm. per 100 ml., with 6 determinations below this level.

Hippuric acid tests of liver function (intravenous) were done on three occasions (Chart 2). The first, on the 28th day, revealed a marked impairment of this function, practically no hippuric acid being excreted during the 1st hour. The result obtained may have been abnormally low because the volume of urine excreted was small. However, even when the 2nd hour excretion was added to the 1st, the total was much less than that normally eliminated in 1 hour. Related to this first examination was an intense and prolonged flush, most disagreeable to the patient, following the injection of the sodium benzoate. This reaction was much less intense with the second test, when the liver function had improved, and was entirely absent with the third, when the test indicated normal function.

The protrombin level on the 13th day was 65% of normal, and on the 65th day, after a period of oral vitamin K and bile salts administration, 100%. A test for serum phosphatase (alkaline) revealed a value of 12 Shinowara units (top normal 8.6) on the 15th day. Subsequent determinations gave results within the normal range.

Duodenal drainage on the 10th day produced specimens containing only traces of bile. Microscopic examination showed innumerable mononuclear cells of an unusual type. The pathologist regarded them as plasma cells. The blood vitamin C level was 0.38 mg. per 100 ml. on the 43rd day (normal 0.4 to 1 mg.). Five days after the daily oral administration of 500 mg. of ascorbic acid, the level was 1 mg. per 100 ml., indicating that the absorption of this vitamin was not significantly impaired.

Dark-field examinations of the blood and urine for *Leptospira icterohæmorrhagiae* were negative, as were the agglutination and complement-fixation tests for antibodies produced by this organism.

A yellow fever virus neutralization test on the serum was carried out through the courtesy of Dr. Max Theiler, of the Laboratories of the International Health Division, Rockefeller Foundation, and no antibodies against the yellow fever virus were found.

Röntgenologic examination of the upper gastro-intestinal tract early in the disease revealed only delayed motility through the small intestine. One month after the disappearance of the jaundice, all of the above tests pertaining to liver function, including a bromsulphalein test, yielded normal results. A cholecystogram has since been done and showed a normally functioning gall bladder without evidence of stones.

Samples of the prehepatic blood of our patient and of the suspected mumps convalescent plasma fortunately are available and have been turned over to appropriate authorities for investigations with reference to the etiologic agent. Injection of these materials into horses failed to reveal any evidence of an agent icterogenic for this animal.\*

*Treatment.* Therapy varied with the course of the disease. A low-fat, high-protein, high-carbohydrate diet, supplemented by multiple vitamins, was administered to the point of tolerance. Vitamin K and bile salts were given orally. The use of barbiturate sedatives was discontinued after the administration of 0.2 gm. of pentobarbital had resulted in a period of somnolence lasting over 24 hours. A mixture of chloral hydrate and sodium bromide was found to be the most satisfactory sedative for this patient. From the 43rd to the 65th day, in spite of what was regarded as an adequate fluid intake by mouth and chiefly because of its reported value in liver disease, an intravenous glucose solution was administered daily, and during the early part of that period, marked improvement, as indicated by a fall in the level of serum bilirubin, occurred. A temporary relapse between the 52nd and the 63rd days led to the use of choline chloride, 1.5 gm. daily by mouth, and whether initiated by this drug or not, progressive improvement was promptly resumed.

\* This work was carried out by Major T. C. Jones, V.C., and Captain Fred D. Mawer, V.C., at the Veterinary Research Laboratory, Army Remount Depot, Front Royal, Va., through the courtesy of Brigadier General Raymond A. Kelsner.

**Discussion.** The differential diagnosis in this case involved the usual question of ordinary catarrhal jaundice (infective hepatitis) *versus* homologous serum jaundice. The fact that the patient had suffered a typical attack of catarrhal jaundice 10 years prior to the recent illness is worthy of note in relation to this problem. A survey of the medical textbooks and recent literature suggests that the occurrence of two separate attacks (not relapses) of infective hepatitis in the same individual is probably uncommon, although Ford<sup>12</sup> records several instances and Lucke<sup>17</sup> has encountered a number of such cases. Cameron, however, states that in Palestine, where the disease is endemic, most authorities agree that one attack confers a permanent type of immunity and that second attacks are unusual. Furthermore, in our patient, the two illnesses were clinically somewhat different. The first began with the typical "grippe-like" prodrome and was associated with fever ranging as high as 102° F. In the second, icterus was the first significant manifestation, no prodromal symptoms having occurred, and fever was conspicuously absent throughout the course of the disease. On the basis of these differences in clinical picture, the history of an injection of pooled mumps convalescent plasma 11 weeks prior to the onset and the absence of any other satisfactory explanation, the diagnosis of homologous serum jaundice seems justified in this case.

The mumps convalescent plasma was obtained from a group of 13 white soldiers, who were convalescing from mumps at one of the army camps in this country. These men were bled in April 1942, and the plasma was pooled and frozen. An attempt has been made to trace these donors, for knowledge of the previous or subsequent occurrence of jaundice in any of them would tend to confirm the diagnosis in our case. Unfortunately, by the time this study was initiated, all but 1 had been transferred to distant theaters and reliable information regarding their medical histories and vaccination records could not be obtained. It was learned, however, that none of the donors had visible jaundice at the time when the blood was drawn. The vaccination records would have been of interest because of the high incidence of jaundice in the United States Army at that time related to the use of certain lots of yellow fever vaccine. It is theoretically possible that one or more of the donors had received one of these icterogenic lots and that the agent was then transmitted through him to our patient. Lacking confirmatory information, the above conception is merely an intriguing hypothesis. Accordingly, this case probably should be regarded, for the time being, as belonging to the group of cases that have been reported as following the use of mumps convalescent serum. In this connection, the fact that the disease has occurred in patients given this type of serum prophylactically indicates that a preceding mumps infection is not necessary for the subsequent development of jaundice.

In view of the data in the literature on water balance in hepatic disorders, it is interesting that in our patient, during a certain stage of the disease, the urinary output, in comparison with the intake of fluid, was high.

Molitor and Pick<sup>22</sup> were among the first to show that water by mouth in the Eck fistula dog resulted in a greater blood dilution and a more prompt diuresis than in the normal. A greater total urinary excretion was also noted. These observations have been confirmed by others and this led Crandall and Roberts<sup>5</sup> to suggest that the increased water exchange is due to a suppression of liver function. Steigmann *et al.*,<sup>28</sup> on the other hand, noting the occurrence of oliguria during the acute stage of hepatic disease, believe that liver damage results in water retention by the tissues and that diuresis is a secondary manifestation indicative of beginning recovery. In our patient, an initial water retention occurred, as manifested by oliguria in spite of an adequate fluid intake; this was followed by a diuresis lasting throughout the period of hospitalization (40 days). The early water retention is in accord with the explanation of Steigmann and his group. The period of diuresis, which occurred without relation to intravenous glucose administration, was, however, much too prolonged to represent merely the excretion of retained tissue fluid and, in this case, could hardly be regarded as a favorable prognostic sign.

Diuresis, therefore, no matter how it may eventually be interpreted or explained, seems to be a manifestation of a certain stage of hepato-cellular disease, and may possibly be related to prolonged suppression of liver function.

A review of the recent literature<sup>15,16,20,24,25,30</sup> pertaining to the fractionation of serum proteins in jaundice suggests that the tendency to low-globulin levels, as found in this case, is unusual. Tumen and Bockus<sup>30</sup> did note the occurrence of low-globulin levels in some cases of acute hepatocellular damage, but no mention was made of this observation in cases of prolonged jaundice. Future cases of serum jaundice, therefore, should probably be studied in this respect in order to determine if this tendency is characteristic of the disease.

The level of serum bilirubin varied inversely and the total cholesterol and cholesterol esters directly with the clinical condition of the patient. This association has been previously observed.<sup>16a</sup>

Pruritus ceased about the 90th day, 40 days after the disappearance of bile salts from the urine, as determined by the methods of Hay and Oliver, but only 5 days after the cessation of bilirubinuria. One wonders about the significance of this relationship in reference to the etiology of pruritus, realizing that the methods used for the detection of bile salts in the urine are not entirely reliable.

The institution of daily intravenous glucose therapy was followed by a decrease in the level of serum bilirubin, an interruption in the steady loss of weight, and a slight increase in the total serum protein. While this improvement may have been incidental, the observations at least suggest that this form of therapy may have been beneficial.

Administration of a homologous blood product probably has been frequently overlooked as an explanation of some cases of obscure jaundice. The duration of the interval between the injection and the resultant jaundice, which is months rather than weeks, doubtless has accounted for the failure to associate the cause and its effect. In an

attempt to find such a relationship, we have found among our records 2 additional cases in point. One occurred in a syphilitic colored woman of 38 years, who developed an afebrile attack of jaundice, with hepatomegaly, that lasted 4 weeks. Her history revealed the administration of 3 blood transfusions for anemia that developed during malarial fever therapy, these having been given 6 to 7 weeks before the onset of jaundice. This patient had also received blood from 2 different malarial blood donors 3 to 4 months before the development of hepatitis, which brings up the possibility of the transfer of an icterogenic agent with therapeutic malarial blood inoculations. The other patient, a 22 year old white woman, had been given 4 blood transfusions for anemia, and 82 days later developed jaundice, hepatomegaly, and slight fever. Beeson<sup>1</sup> and others have regarded similar cases as due to an agent in the injected blood. At present, however, such a case can be differentiated from infective hepatitis only by the interval of 1 to 3 months that usually elapses between the injection and the onset of jaundice.

**Summary.** 1. A case of severe and prolonged hepatocellular jaundice that followed the injection of pooled mumps convalescent plasma is reported and represents a typical example of a problem that occasionally may be encountered as a result of the increasing use of human blood and its derivatives in modern therapeutics. The question of similar hepatocellular jaundice without administration of homologous blood has not been considered in this report.

2. The features of homologous serum jaundice as reported in the literature are reviewed.

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## BLOOD STUDIES IN ALLERGY

### II. THE PRESENCE IN ALLERGIC DISEASE OF ATYPICAL LYMPHOCYTES AND SYMPTOMS SUGGESTING THE RECOVERY PHASE OF INFECTIOUS MONONUCLEOSIS\*

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In performing differential white cell counts in patients with allergic disease, one of us (E.B.G.) noted the frequent occurrence of large mononuclear cells indistinguishable from the atypical lymphocytes seen in infectious mononucleosis. The number of such cells was comparable to the blood findings in the subsiding phase of infectious mononucleosis, but distinctly less than that observed in the active stage of this disease.

We then observed that several patients under study for allergic disorders had previously been suspected of having infectious mononucleosis. The finding of atypical lymphocytes had aroused this suspicion in the majority of instances. In others, the general symptoms of debility or the presence of a cervical adenopathy had suggested the diagnosis. It is of interest that both allergic disease and infectious mononucleosis commonly occur during early adult life in patients otherwise in the best of health.

Weakness, lassitude, headache, or general malaise are often the outstanding symptoms in infectious mononucleosis. Not infrequently the weakness and related symptoms may persist for many months following the acute stage of the disease. Although these symptoms are well recognized as part of the clinical picture of infectious mononucleosis, their presence in clinical allergy has not been sufficiently emphasized. Rowley<sup>14</sup> in 1929 described "allergic toxemia" resulting from long-standing food allergy. According to him, "This toxemia produces drowsiness, mental confusion, slowness of thought, difficulty in remembering and concentrating, lack of initiative and ambi-

\* This study was financed in part by the Parke, Davis Company.

tion, irritability, despondency, fatigue, weakness, bodily aching, and a feeling of "being poisoned". He found these symptoms more frequently in women than in men and noted their prompt disappearance after dietary management. Vaughan<sup>2</sup> mentioned the "droopy, lousy, no 'count feeling" resulting from the ingestion of an allergenic food. Similar symptoms in patients with food allergy manifestations have been observed by others.<sup>3,12</sup>

The present study deals with a series of 24 allergy patients having fatigue or weakness as a major complaint. The fatigue in these cases was unrelieved by rest, even though more than an average amount of sleep was obtained nightly. Some patients complained of being more tired in the morning than when they had gone to bed in spite of receiving 10 to 12 hours of sleep. The characteristic drowsiness and inertia were particularly troublesome during the first few morning hours, but sometimes persisted throughout the day and not uncommonly were present daily for weeks or months. In some cases, drowsiness to the point of sleepiness was more prominent 1 to 2 hours after a meal containing allergenic foods. Certain students, at the time of allergic symptoms, found it difficult to remain interested and attentive during the school day, and during the evening were overcome by a compelling sense of fatigue and sleepiness. "Fuzzy thinking" and the inability to concentrate and to remember details proved a distinct handicap in certain instances. Some patients complained of being "dragged out" and lethargic to a degree that it required a distinct effort to carry out their ordinary daily routine. Others had generalized aching, apparently similar to the deep aching sensation associated with the rapidly dropping leukocyte count in the early stages of agranulocytosis due to drug allergy.

Such symptoms often appeared in association with allergic rhinitis, bronchial asthma or some other easily identified allergic manifestations, but when occurring in connection with some less readily recognized allergic expressions, such as the migraine type of headache or certain gastro-intestinal symptoms, the underlying allergic mechanism was frequently not appreciated. This was particularly true when the fatigue or lassitude represented the major complaint at the time the patient was seen, although the past history may have been replete with evidence of allergic disease. A history including details of all probable allergic manifestations occurring from infancy on, including "well focused" questions regarding the major food, drug, and inhalant allergens, will serve to detect many of these cases.

The fatigue, drowsiness, and related symptoms in this series of patients appeared most commonly as the result of allergic intolerance to foods, our experience in this respect confirming the earlier observation of Rowe. Such symptoms have been observed repeatedly after the trial ingestion of foods in susceptible patients, occurring both in association with, and not infrequently in the absence of, other evidence of allergic intolerance.

In the series studied, each patient had at least one clinical allergic manifestation in addition to his complaint of fatigue. As may be

TABLE 1.—ATYPICAL LYMPHOCYTES IN 24 ALLERGIC PATIENTS

Case	Age	Sex	Diagnosis (see key)*	Proylene glycol stain			Differential count, Wright stained film (%)										Downey's classif. atypical lymphs.	Heterophili antibody				
				Total white blood count (thous./ cmm.)	Neutro- phils	Eos.	Mono- nuc- leus	Neutro- phils	Eos.	Bas.	Nor- mal lymph.	Atypi- cal lymph.	Mono- cytes	Smudges	1/4	1/8		1/16	1/32	1/64		
R. P.	29	M	4, 5	5.6	55	5	40	52.0	5.5	0.5	14.5	17.5	1.5	8.5	111	++	++	+	+	0		
D. J.	23	F	1, 2, 3, 5	7.1	64	1	35	59.0	3.0	0.5	13.5	10.0	2.5	7.0	111	++	++	+	+			
D. D.	26	F	2, 5, 6	8.3	68	2	40	50.5	2.5	0	22.0	10.5	3.0	6.0	111	++	++	+	+			
W. B.	29	F	4	6.4	70	2	28	71.0	0.5	0.5	9.0	8.5	6.0	4.5	111	++	++	+	+			
M. P.	37	F	1	6.4	76	0	24	69.5	0.5	0.5	14.5	7.5	4.5	3.0	111	++	++	+	+			
F. H.	39	M	4	9.0	54	0	45	53.0	1.0	0.5	14.0	12.5	4.5	14.5	111	++	++	+	+			
N. W.	27	M	4	7.3	53	12	35	55.5	3.5	0	27.0	8.0	3.5	8.5	111	++	++	+	+			
D. S.	23	M	1, 3, 6	7.1	60	10	30	60.5	10.5	1.0	27.5	11.0	1.0	22.5	111	++	++	+	+			
S. Nt.	23	F	3, 4, 5	4.5	56	3	41	43.5	3.5	1.5	21.5	7.0	3.0	7.0	111	++	++	+	+	0		
J. S.	29	F	2, 4, 5	7.7	55	4	38	50.0	8.0	0	27.0	13.0	4.5	8.5	111	++	++	+	+			
S. L.	24	F	4, 5	6.7	51	11	34	58.0	1.5	1.5	11.0	13.0	1.5	9.0	111	++	++	+	+			
V. B.	20	M	5, 4, 5, 6	8.5	46	20	34	53.0	18.5	0.5	20.0	9.5	2.5	8.5	111	++	++	+	+			
H. M.	27	M	4, 5, 6	6.0	70	5	24	77.0	4.5	1.0	20.5	2.0	4.0	1.5	111	++	++	+	+			
W. H.	21	M	4, 5	4.6	63	5	32	77.0	4.5	1.5	13.0	2.0	0.5	3.0	111	++	++	+	+			
N. D.	25	F	4, 5, 6	5.9	62	11	27	65.0	4.5	0.5	20.5	10.5	3.5	3.0	111	++	++	+	+			
I. K.	23	F	4	4.7	61	1	38	68.0	1.5	0.5	16.0	4.5	1.0	4.5	111	++	++	+	+			
G. R.	34	F	2, 3, 5, 6	4.0	69	1	30	59.0	1.0	1.0	17.5	15.0	1.0	9.0	111	++	++	+	+			
L. F.	28	F	4, 4, 4, 4, 4	4.4	50	5	45	42.0	11.0	1.0	21.0	14.0	5.0	9.5	111	++	++	+	+			
I. H.	35	M	2, 3	5.3	57	8	35	41.0	5.0	0	21.0	21.0	3.0	9.0	111	++	++	+	+			
E. R.	36	F	2, 3	6.2	64	7	29	64.0	1.5	0.5	18.5	9.0	2.0	4.5	111	++	++	+	+			
D. M.	21	M	2, 5	10.4	77	5	18	61.0	7.0	0.5	13.5	10.0	5.5	2.5	111	++	++	+	+			
V. E.	22	M	2, 5	7.2	62	1	37	68.0	1.0	0.5	13.0	9.0	3.0	3.0	111	++	++	+	+			
V. B.	27	F	2, 5	6.5	65	1	34	70.5	4.5	0.5	13.5	9.0	1.0	1.0	111	++	++	+	+	0		

\* 1, Atopic eczema; 2, allergic rhinitis; 3, bronchial asthma; 4, allergic headache; 5, gastro-intestinal allergy; 6, urticaria.



observed from the accompanying table, females of early adult type were in the majority. Many were nurses or persons of the hospital personnel who had been under careful medical supervision for an extended period. In 8 of the 25 cases, there was definite evidence of cervical gland enlargement, present at the time of the allergic study or observed upon some past occasion not readily explained.

Total and counting chamber differential leukocyte determinations were performed by the use of propylene glycol-aqueous stains, previously described.<sup>9,10,11</sup> A differential count of 200 cells from the standard Wright-stained cover slip and heterophil antibody determinations by the method of Paul and Bunnell<sup>12</sup> were done.

The atypical mononuclear cells observed in the films of these cases are similar morphologically to the 3 types of lymphoid cells in infectious mononucleosis as described by Downey.<sup>4</sup> Cells corresponding to his Type III are encountered most frequently. This cell has a very large nucleus, approximating the size of the nucleus seen in the blast forms of acute lymphatic leukemia, but differing from the latter in that the chromatin strands are more pronounced. Instead of the pale, evenly marked nucleus of the stained immature forms, the nucleus of this cell is more mottled due to slightly more clumping of the chromatin material. There are many sharply defined pale spots in the nuclei, unlike nucleoli, but suggesting holes through the nucleus. This feature has been described by Osgood<sup>13</sup> as "nuclear fenestrations." Nucleoli also appear occasionally.

Although the nucleus is invariably large, the amount of cytoplasm varies. The presence of many vacuoles in the cytoplasm of the larger cells gives them a characteristic foamy appearance. When less in amount, the cytoplasm is apt to be concentrated in a basophilic rim which is separated from the nucleus by a relatively clear zone. Although azurophil rods have not been observed, there often is an abundance of large, deeply stained azurophil granules and occasionally fine, azure granulation.

The Type II cells are slightly less common, but they occur in all the variations described by Downey. In some instances the nuclei are marked with several dense and sharply defined rounded masses of chromatin. The most common form (Downey's cell No. 7) contains a large irregular nucleus, appearing less immature than the nucleus of the Type III cells. The cell outline is angular and edges of the cytoplasm are irregularly basophilic. At times, only the points appear basophilic. Downey's Type I cells were seen least frequently. These cells are relatively smaller than those of Types II and III. The nucleus is similar in size to that of the mature lymphocyte and may show marked lobulation; the chromatin exists in the form of a coarse network of heavy strands and masses. The cytoplasm is very basophilic and contains many vacuoles. Azure granulation is common and there is an abundant hyaloplasm.

The total number of mononuclear cells does not exceed the polymorphonuclears in any instance, as determined by either method of differential counting. The atypical lymphocytes range from 2 to 27%

of the total differential count. In several cases, the differential count was repeated at various times; atypical mononuclear cells were always observed, although the total number varied. In a small group of cases, serial counts were determined prior to and following the ingestion of foods known to produce allergic symptoms. Variations which occurred were inconstant and were not regarded as significant in view of the recognized errors in the distribution of large cells which occur in the preparation of the blood film.

Toxic granulation of the neutrophils accompanies the presence of atypical mononuclear cells in the majority of the films. This, incidentally, is a common finding in infectious mononucleosis. Identical hematologic technique in a control series of 20 normal non-allergic individuals of similar age and sex distribution failed to reveal a significant number of atypical lymphocytes.<sup>9</sup>

Heterophil antibody determinations were normal in all instances in which the test was performed.

**Discussion.** The question is immediately raised whether the patients in this series might not be convalescing from infectious mononucleosis. Only one case (V.B.) was known to have had a past diagnosis of infectious mononucleosis. Two years earlier, her white blood count was recorded as 6000 cells, and the differential showed 28% neutrophils, 58% lymphocytes, 13% monocytes, and 1% basophils. The heterophil antibody reaction was strongly positive in a dilution of 1/256 and slightly positive to 1/512. She had been subject to hay fever for many years, and at the time studied was experiencing gastro-intestinal symptoms of nausea, abdominal cramps and diarrhea which were relieved with milk avoidance and were precipitated at will by the ingestion of milk.

Seven of the remaining cases had been suspected of having had infectious mononucleosis in the past, but their heterophil antibody determinations had been consistently negative.

It is well known that abnormal lymphoid cells remain in the blood for appreciable periods after the active phase of infectious mononucleosis has subsided, and that the presence of abnormal lymphocytes in the routine blood examination may be evidence of infectious mononucleosis months or even years previously.<sup>1,2</sup> There is one case on record in which atypical cells characteristic of infectious mononucleosis occurred 10½ years after the original illness.<sup>4</sup>

We cannot state that the remaining 23 cases of this series had never had infectious mononucleosis, but this would appear unlikely, in view of the frequency with which we have observed these findings in cases of clinical allergy.

It is also well known that the presence of atypical lymphocytes in the peripheral blood is not diagnostic of infectious mononucleosis; such cells may occur in association with other disease processes. Warren<sup>17</sup> has recently reported finding cells characteristic of this disease, comprising between 1 and 10% of the differential count, in cases with upper respiratory infections, acute pharyngitis, or sinusitis. He also reported a group of cases having the clinical findings

of infectious mononucleosis with abnormal lymphocytes ranging up to 25% who lacked a significant heterophil response. The tendency of abnormal lymphocytes to enter the blood stream in cases with lymphoid hyperplasia other than infectious mononucleosis was pointed out by Baldridge, Rohner, and Hansmann.<sup>15</sup> The presence of atypical lymphocytes not associated with infectious mononucleosis has also been observed by others.<sup>16</sup>

**Summary.** 1. The laboratory finding of atypical lymphocytes in the peripheral blood of patients with clinical allergy not infrequently suggests the diagnosis of infectious mononucleosis. The number of such cells is compatible with the blood findings in the recovery phase of this disease.

2. The symptoms of weakness, fatigue, and lassitude frequently characterize the allergic reaction, and commonly occur as sequelae to acute infectious mononucleosis.

3. The fact that both conditions frequently occur in young adults otherwise in good health adds to the difficulties of differential diagnosis.

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## RELATIONSHIP OF LYMPHOGRANULOMA VENEREUM INFECTION TO THE INCIDENCE OF HYPERGLOBULINEMIA

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An increase in the globulin fraction of blood serum is known to occur in certain diseases, among which are cirrhosis of the liver, lympho-granuloma venereum, sarcoid, multiple myeloma, kala azar, and some

acute infections. There is little information available regarding the general incidence of hyperglobulinemia, although this undoubtedly varies in different parts of the world. In Denmark it is apparently rare, as Bing<sup>1</sup> found only 7 instances of increased globulin among 3697 sera which had been submitted to a laboratory in that country for serologic tests for syphilis; this is an incidence of less than 0.2%. In localities where kala azar or lymphogranuloma venereum are prevalent hyperglobulinemia is undoubtedly more common. The present study is concerned with the incidence of hyperglobulinemia in residents of Georgia. Our interest in this problem arose from observations made in a serologic study of the frequency of lymphogranuloma venereum infection in clinic patients. The findings in that study, which will be presented in detail elsewhere, are in accord with the general concept that lymphogranuloma venereum is far more common among negroes than among white people, and also that immunologic evidence of infection can often be obtained in the absence of a history or clinical sign of the infection. One finding which supported the specificity of the serologic test is the fact that many of the positive sera were also found to have an increased globulin content. Because we had found hyperglobulinemia to be relatively common among the patients of one hospital it seemed of interest to determine the incidence of the condition in a less selected sample of people in the same locality. Accordingly we have carried out formol-gel tests on 2375 sera which had been submitted to the Central Laboratory of the Georgia Department of Public Health, for serologic tests for syphilis. The results are the subject of this paper.

**Material.** The specimens used had been sent by mail to Atlanta from various parts of Georgia. Some of them were from patients of private physicians or of clinics, while others were submitted as part of routine institutional surveys or examinations for military service. This group of sera cannot be called a random sample from the population in this area, but is nearer to a random sample than could be obtained in any other way. Our material is perhaps comparable to that of Bing<sup>1</sup> in Denmark. **Methods.** Any serum which appeared cloudy, or which showed evidence of hemolysis, was rejected. Hyperglobulinemia was detected by means of the formol-gel test, in which a positive reaction indicates that the level of globulin is above 3.5 gm. per 100 cc. of serum.<sup>2</sup> Each test was set up by adding 2 drops of 37% formaldehyde to 1 cc. of serum in a 13 x 130 mm. test tube. The tube was stoppered, its contents mixed by inverting, and then allowed to stand at room temperature for 24 hours. Interpretation of the reaction was based only on gel formation, no account being taken of the development of oily consistency or cloudiness. The result was read as positive if the serum had gelled to such extent that more than half remained in the bottom of the tube upon inversion. The complement-fixation test for lymphogranuloma venereum was done according to the technique of McKee *et al.*,<sup>3</sup> using as antigen the commercial egg yolk preparation, Lygranum C.R. (Squibb).

**Results.** The incidence of hyperglobulinemia, in relation to the race, sex and age of the persons from whom the blood specimens had been obtained, is shown in Tables 1 and 2. The most prominent finding is the marked racial difference: hyperglobulinemia was more than 10 times as frequent in the sera of negroes as in white persons.

TABLE 1.—INCIDENCE OF POSITIVE FORMOL-GEL REACTIONS, IN RELATION TO SEX AND AGE GROUP, IN THE SERA OF 1307 WHITE PERSONS

Males		Females	
No. of sera positive	%	No. of sera positive	%
22	0	15	0
183	0	393	2
98	0	253	1
84	1	122	0
62	1	75	2
449	2	858	5
Total			0.6

TABLE 2.—INCIDENCE OF POSITIVE FORMOT-GEL REACTIONS, IN RELATION TO SEX AND AGE GROUP, IN THE SERA OF 1068 NEGROES

Males		Females	
No. of sera	%	No. of sera	%
22	13.6	37	0
174	5.7	265	20
188	4.3	152	18
73	2.7	78	4
46	8.7	33	5
503	28	565	47
Total		Total	

We believe that a difference in the prevalence of infection with lymphogranuloma venereum must be directly related to the racial difference in incidence of hyperglobulinemia. All studies, including our own, indicate that this disease is much more frequent in negroes. Other causes of hyperglobulinemia, such as liver disease, sarcoid and multiple myeloma do not appear to manifest any such racial affinity; furthermore none of them is sufficiently common to affect 5 to 8% of the general population at one time.

In regard to sex incidence, hyperglobulinemia was found in a slightly higher proportion of females than of males. The total number of positive sera from white people was so small that this difference cannot be regarded as significant; but in the case of the negroes, where increased globulin was relatively common, the higher incidence found in females may be significant—perhaps it is a reflection of the different forms which the disease assumes in females and males.

In Table 3 the incidence of hyperglobulinemia is compared with the result of the serologic tests for syphilis. This shows that increased globulin was more frequent in the sera of persons with syphilis. (The data on white persons are not included because there were only 93 Kahn-positive sera from white persons; 1 of these gave a positive formol-gel test). One might infer from the data in Table 3 that syphilis is a frequent cause of hyperglobulinemia, however, there is

considerable evidence available indicating that such is not the case. A more probable explanation of the relationship is simply that persons who have acquired one venereal disease are apt to have acquired another. This view is supported by the results which we obtained when some of the same sera were tested for complement fixation with lymphogranuloma venereum virus. One hundred and thirty Kahn-positive sera, all from negroes, were tested. In 5 of them the result could not be determined because of reaction with the antigen control, a difficulty which is encountered occasionally in syphilitic sera.<sup>5</sup> Of the remaining 125 sera, 74 gave a positive test for lymphogranuloma venereum, while 51 were negative. In these 2 groups there were 13 cases of hyperglobulinemia; 12 of them were from persons with lymphogranuloma venereum, while only 1 was from a person with syphilis alone. This appears to be a significant difference, and points to lymphogranuloma venereum rather than to syphilis as the principal cause of hyperglobulinemia.

TABLE 3.—INCIDENCE OF POSITIVE FORMOL-GEL REACTIONS, IN RELATION TO RESULT OF KAHN TEST FOR SYPHILIS; SERA FROM 1068 NEGROES

Kahn-positive				Kahn-negative			
No. of sera		Formol-gel positive		No. of sera		Formol-gel positive	
%		%		%		%	
0-14	5	1	20.0	54	2	3.7	
15-24	169	16	9.5	270	14	5.2	
25-34	206	23	11.2	134	4	3.0	
35-44	84	5	6.0	67	1	1.5	
45 and over	21	4	19.0	53	5	8.6	
Total	485	49	10.1	583	26	4.4	

**Summary.** Because of the prevalence of lymphogranuloma venereum in the negro population in Georgia, a study was made of the incidence of hyperglobulinemia in 2375 sera, from both the white and the negro population. The formol-gel test was used to detect the presence of increased globulin. Hyperglobulinemia was found in 0.4% of white males and in 0.6% of white females. In negroes the respective rates were 5.6% and 8.3%. The difference in prevalence of lymphogranuloma venereum infection is presumed to be the explanation of this racial difference. A higher proportion of cases of hyperglobulinemia found among negroes with syphilis appeared to be related to the associated presence of lymphogranuloma venereum in those persons.

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# CHLOROPHYLL: AN EXPERIMENTAL STUDY OF ITS WATER-SOLUBLE DERIVATIVES\*

## I. REMARKS UPON THE HISTORY, CHEMISTRY, TOXICITY AND ANTIBACTERIAL PROPERTIES OF WATER-SOLUBLE CHLOROPHYLL DERIVATIVES AS THERAPEUTIC AGENTS

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In recent years interest in the possible therapeutic value of various chlorophyll derivatives has been revived, after a hiatus of nearly a quarter of a century. Up to the present time, however, such evidence as has appeared in the literature has been so relatively scanty and inadequately controlled, that it seemed worth while to attempt to explore certain aspects of the problem by well established experimental methods in an attempt to evaluate its potentialities clinically. To this end, a number of experiments were designed and carried out to determine certain more or less specific facts regarding the nature and manner of action of therapeutic preparations made with stable, purified, water soluble chlorophyll derivatives.†

In the first place, it seemed important to establish whether or not the chlorophyll derivatives were toxic. Second, studies regarding the bactericidal or bacteriostatic effects of these derivatives seemed definitely indicated, in view of the favorable reports which had appeared in respect to its benefits in a wide variety of infectious conditions. Third, the effect of chlorophyll as a growth stimulating agent, in comparison with other substances in current use or under investigation at the present time in the repair of wounds of both traumatic and thermic origin, seemed of particular importance to ascertain, in view of its very great practical interest in the war program. In addition to these 3 main aspects of the problem, it was hoped that incidental accumulated data from these studies might yield further information as to the mechanism of the action and fate of chlorophyll in the body and any possible effect it might have upon the hematopoietic system through its close structural composition to that of hemoglobin.

### Chemistry of Chlorophyll.

Chlorophyll, the green coloring matter of plants, has stirred the imagination of biologists through the centuries. It is perhaps but natural that the greatest interest has been exhibited

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† The experimental products were generously supplied us by the Rystan Company of New York City, sole licensee of the Lakeland Foundation, Chicago, in accordance with the regulations of the Food and Drug Administration regarding new experimental therapeutic agents.

‡ However the term "chlorophyll" is used in these studies the water-soluble derivatives are meant, the term "chlorophyll" being used for the sake of brevity.

§ I am indebted to Prof. Paul Rothmund of Antioch College and to Isidor Chamein, M.S., of the Goldwater Memorial Hospital, Welfare Island, New York City, for their kindness in reviewing and revising the section relating to the chemistry of chlorophyll.

by those scientists concerned particularly with plant physiology, and above all, by the biochemists, who have sought to understand the complex mechanism of the conversion of carbon dioxide to starch by plants through photosynthesis and the action of chlorophyll. Even among the ancients, the greenest herbs were chosen for their crude empiric remedies. But it was not until Willstätter and Stoll's<sup>23</sup> (1913) monumental studies upon chlorophyll that its empirical formula was established. Since that time, the further studies of Stoll,<sup>19</sup> Conant, Hans Fischer,<sup>6,7</sup> and others<sup>1,5,9,12-15,18,20-22</sup> have established the molecular formula (see Fig. 1). The unfolding of our knowledge regarding the chemistry of chlorophyll will be found in the publications listed in the appended bibliography.

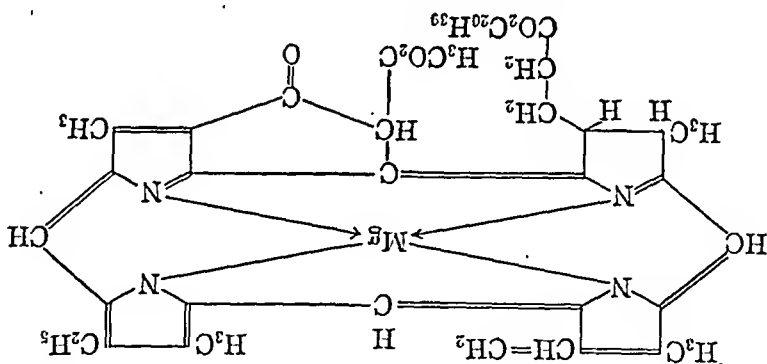
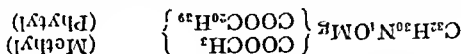


Fig. 1.—The structural formula for chlorophyll *a*, as determined by Hans Fischer.<sup>7</sup>

Chlorophyll, from present knowledge, consists of two components: chlorophyll *a* (C<sub>55</sub>H<sub>72</sub>O<sub>5</sub>N<sub>4</sub>Mg) and chlorophyll *b* (C<sub>55</sub>H<sub>70</sub>O<sub>6</sub>N<sub>4</sub>Mg). These components occur in nature in an almost invariable proportion of 2.9:1. They are relatively inert blue-black substances with a marked metallic luster. They have no definite melting points, varying from 117° to 120° C. for the *a* form and 120° to 130° C. for the *b* form. Both forms are optically active: the specific rotation of the *a* form is at -262° and of the *b* at -267°. The two fractions are neutral in reaction, with neither acidic nor basic properties.

Examination of the structural formula of chlorophyll *a* (Fig. 1) as determined by H. Fischer<sup>7</sup> (1939) shows the substance to be a diester:



The phytol group is placed on the propionic acid residue of pyrolic nucleus IV while the carbmethoxy group is attached to the isocyclic ring (C<sub>10</sub>).

The formula of chlorophyll *b* as it is known at present, has an aldehydic or formol group on C<sub>3</sub> in place of the -CH<sub>3</sub> of chlorophyll *a*. This substitution yields 2 hydrogen atoms less and 1 oxygen atom more than in the chlorophyll *a* structure.

Treatment of either chlorophyll with mild acids, such as oxalic acid, removes the nuclear magnesium. More vigorous acid action removes the phytol radical in addition.



The degradation products run parallel in the *a* and *b* components forming pheophytins *a* and *b*; pheophorbides *a* and *b*; chlorins from *a* and rhodins from *b*. The porphyrins and further degradation compounds are the same whether obtained from chlorophylls *a* or *b*. The chlorophylls are easily soluble in absolute ethyl alcohol, somewhat less soluble in 95% ethyl alcohol as well as in methyl alcohol. They are not soluble in petroleum ether.

Chlorophyll may be obtained from either fresh or dried leaves by extraction with acetone. It occurs in greatest concentration probably in nettes, alfalfa and spinach. A kilo of fresh leaves yields about 1.5 gm. of chlorophyll on the average, while about 7.5 gm. can be obtained from a kilo of dried leaves. In its natural state it occurs in colloidal form. As isolated from plants it is amorphous in character (phytylchlorophyllide), but it can be secured in crystalline form (methyl or ethyl chlorophyllide) through the substitution of the phytyl radical by the respective alcohol either through chlorophyllase enzymic action or by alcoholic extraction.

The similarity of hemoglobin and chlorophyll is seen in the comparison of the hematin and chlorophyll molecules as described by Gortner.<sup>9</sup> In hemoglobin, iron is substituted for the magnesium of chlorophyll, globin is substituted for the phytyl radical, and at Carbon 4 an allyl instead of an ethyl residue is obtained.

The water-soluble chlorophyll used in these experimental studies, as supplied by the Rystan Company, is a medicinally purified product, from which the resins and other impurities have been removed as far as possible by adsorption. In the main we used a saponified metal complex derivative of chlorophyll, such as sodium copper chlorophyllin. It has been provided in two forms: (1) as saline solutions in strengths ranging from 0.2 to 5%, and (2) as ointments in lanolin, cholesterol and hydrophilic bases. Both types of product as used experimentally and clinically, have had a preservative, propyl-para-hydroxybenzoate, added in small amounts; but for bacteriologic and tissue culture experiments, sterile material has been given us without such added preservative. The pH of the solution has varied also in the products for clinical use—that for nose and throat work being buffered to approximately pH 6.7, while the ordinary, unbuffered solution has had a pH which ranged from 7.3 to 8.5. The standard ointment has contained 1% of the water-soluble chlorophyll although earlier preparations varied in their chlorophyll content from 0.25% to 3% in an attempt to find the optimal concentration.

Most previously published clinical studies have been concerned with the oil-soluble product.<sup>2,3,12</sup> Experience over a period of years has convincingly demonstrated that the water-soluble derivatives are much preferable in clinical use, as they are equally, if not more effective,<sup>10</sup> than the oil-soluble form and at the same time are bland and non-irritating. The oil-soluble fraction also presents certain obvious objections to its use: it does not lend itself readily to use on mucous membranes; it is difficult to obtain in pure form, and it tends to be slightly irritating, presumably due to impurities. For these reasons, we have elected to use aqueous-soluble products in these studies.

**Toxicity Studies.** In a series of 20 rabbits—chlorophyll (provided as a 0.2% saline solution with a pH of 7.3) was administered: (a) by mouth, (b) intravenously, (c) intraperitoneally and (d) subcutaneously, separately and in combination. It has likewise been given in a smaller series of 5 rabbits (a) by mouth and (b) intravenously in concentrated form (2%).

**Method.** The dosage has varied from 5 cc. subcutaneously to as much as 150 cc. intravenously at one time. The majority of the animals have been given the drug intravenously and in repeated dosage. The usual interval was 5 days and the average single dose was 50 cc. In 2 animals this amount was administered daily for 5 days. One animal received a total of 550 cc. in 8 doses at 5-day intervals, the first 6 being 50 cc., the 7th dose 100 cc., and the 8th dose 150 cc. In several of the animals, as a purely side observation, the effect upon hematopoiesis was noted by removing a corresponding volume of blood before the drug was given, and following the blood count at intervals.

None of the animals given chlorophyll in any of the experiments, regardless of the dosage, showed any systemic reaction indicative of toxic effect. There was no elevation of temperature and no significant gastro-intestinal symptomatology other than a slight diarrhea in an occasional instance. Their appetite was not affected, nor was their physical activity. The red cell count returned to normal within 4 or 5 days in these animals which were bled as much as 50 cc. repeatedly. This would suggest that the drug might perhaps have some actual stimulating effect upon hematopoiesis.

Pathologic examination of several of the animals show considerable retention of the pigment in the reticulo-endothelial system, notably the spleen and bone marrow. Whether this might eventuate in reticulo-endothelial block under such extreme experimental dosage cannot be answered at this point; but, as reported by Henderson and Long<sup>11</sup> as well as in our experience, there is no evidence to suggest that this does occur. Our impression, at present, is rather that any effect which takes place is in the nature of cell stimulation.

The chlorophyll solution likewise has been given intravenously to a number of dogs, cats, guinea pigs, rats and mice with essentially similar negative results so far as any toxic effect is concerned. No attempt has been made in these animal experiments to evaluate the drug therapeutically in any way. They were concerned solely with an investigation of its toxicity.

Clinically, 240 cc. of the 2% solution has been given daily for 3 days by mouth to a normal healthy adult male volunteer. No subjective or objective symptoms or signs occurred other than a slight increase in bowel evacuation, the stool being somewhat softer than normal, and somewhat discolored by the drug. In addition, as much as 500 cc. of 0.5% chlorophyll solution has been administered intravenously daily for 8 days in cases of subacute bacterial endocarditis with no toxic symptoms, and with some temporary bacteriostatic effect. As regards toxicity, we can conclude without qualification that such medicinal water-soluble chlorophyll derivatives as are contained in the preparations we have employed in these studies are without any toxic effect upon the usual laboratory animals or on man.

**Bacteriologic Studies.\*** In view of the striking results, as reported by several clinics<sup>2,3,8</sup> and numerous doctors who have investigated these same water-soluble chlorophyll preparations during the past 3 or more years, in reducing or eliminating the infections associated with many ulcerative lesions, both clinical and as induced in experimental wounds in animals,<sup>4</sup> it was felt that a survey of the antibacterial properties of chlorophyll, being derived from plants, and possessing certain cellular growth stimulating properties as demonstrated in tissue culture<sup>7</sup> and wound healing experiments,<sup>16</sup> would tend similarly to accelerate rather than inhibit bacterial growth. Yet the evidence seems to indicate that chlorophyll exerts a definite bacteriostatic effect upon many of the common tissue invaders; and even an actual bactericidal effect upon some of the more delicate organisms, notably Lancefield Type A hemolytic streptococci and some of the anaerobic bacteria. In this connection Henderson and Long<sup>11</sup> have shown recently that "phytorhodin g" has a definite inhibitory effect upon the growth of tubercle bacilli in culture, but that it does not materially alter the course of the experimentally induced disease in guinea pigs, although it tends to localize the extent of the process.

Preliminary studies revealed that chlorophyll alone in dilutions of from 1:100 to 1:500 would not support the growth or even the viability of staphylococci, streptococci or coliform bacteria over a period of 24 to 48 hours. Dilutions of chlorophyll up to 1:2000 in a brain heart broth medium showed a definite bacteriostatic effect upon the growth of these organisms although growth was not completely inhibited in this medium beyond a 1:500 dilution.

**Method.** On the basis of the results of these preliminary experiments somewhat more extensive tests were made, using beef infusion broth as the medium, and increasing the inoculum to average 90,000 organisms.

1. *Staph. aureus* was inhibited by 1:80 dilution, but not by 1:160. It was not killed by 24 hour exposure to 1:20 dilution.
2. *Strep. hemolyticus* was inhibited by 1:640 dilution, but not by 1:1280. It was killed also by the 1:640 dilution.
3. *Ksch. coli* was not inhibited by the 1:20 dilution.
4. No evidence of morphologic variation noted except a tendency for the streptococci to become gram negative after 7 days.

It was felt wise to extend these bacteriologic studies a little further to include certain of the anaerobic organisms and *Ps. pyocyaneus*. The prompt deodorization and cleaning up of infected, ulcerated lesions, such as carcinoma and varicose ulcers, clinically, often within a matter of 48 to 96 hours, prompted this line of attack in an effort to attempt to explain the action of chlorophyll in such cases.

In these experiments 8 organisms were employed, varying the amount of the inoculum, and the type of media. The organisms used were: 1. *Staph. aureus*, a highly hemolytic, coagulase positive strain fermenting lactose and mannite, No. 209.

\* The bacteriologic studies were carried out through the cooperation and supervision of E. H. Spaulding, Ph.D., Associate Professor of Bacteriology.

2. *Strep. hemolyticus*, Group A—recent isolation.
3. *Strep. hemolyticus*, Group A—strain C-203.
4. *Strep. mastitidis* (agalactiae), Group B—recently isolated from an infected herd of cattle, through the courtesy of Dr. Oscar Frit of Ohio State University.
5. *Ps. pyocyanus*, a recent isolation.
6. *Esch. coli*, freshly isolated hemolytic strain.
7. *Cl. perfringens*, a Sharp & Dohme strain No. 1633.
8. *Cl. histolyticum*, A. T. C. C. No. 6282.

Initially, veal infusion, and 1% amigen broth were employed. The inoculum averaged about 9000 colonies except in the case of the two anaerobes in which it was only about 1000 colonies. Inhibition of growth of *Esch. coli* and *Ps. pyocyanus* was noted in 1:50 dilutions; of *Strep. mastitidis* in 1:200 dilution, of the streptococci, and the two anaerobes in 1:1600 dilution and no inhibition of the hemolytic staphylococcus was obtained. The experiments were repeated increasing the inoculum with approximately the same results.

This apparent bactericidal effect of chlorophyll upon anaerobic bacteria as suggested by these last outlined experiments could be explained as dependent upon an oxidizing mechanism. To test this hypothesis, these tests were repeated using this time, however, (Brewer's) thioglycollate medium which does not require the use of an anaerobic jar, and which contained as a constituent, an oxidation-reduction indicator, methylene blue. Control experiments using the medium alone with the addition of chlorophyll in dilutions of from 1:10,000 to 1:40,000 were made. The results indicated that chlorophyll was unable to oxidize the thioglycollate medium appreciably, and its inhibiting effect upon the growth of *Cl. perfringens* and *Cl. histolyticum* was sharply reduced, being ineffective in dilutions of 1:500.

These results were in such striking contrast to those obtained earlier with veal infusion broth that it was thought advisable to repeat the earlier experiments adding a reducing agent, cysteine hydrochloride, to the veal broth medium. This had a final concentration of 0.02%. Again it was demonstrated that chlorophyll was unable to prevent the reduction of the methylene blue in the presence of such small amounts of cysteine hydrochloride, and that the inhibition of anaerobic bacterial growth was strikingly reduced, although in this case stasis was obtained for 48 to 72 hours in dilutions of 1:500 and 1:1000. These studies suggest that some interference with the oxidation-reduction mechanism of bacterial respiration is produced by the action of chlorophyll, which might well account for its bacteriostatic and even bactericidal effect clinically in a large proportion of cases.

**Discussion.** Studies on the effect of water-soluble chlorophyll preparations on cultures of various of the more common pathogenic bacteria tend to support the premise that chlorophyll acts to produce an unfavorable environment for bacterial growth rather than by any direct action upon the bacteria themselves. However, it is to be noted that the ordinary pyogenic bacteria will neither grow nor survive for 24 hours in simple isotonic saline solutions of chlorophyll up to dilu-

tions of 1:500.

The experiments point towards an oxidation mechanism as perhaps most readily explaining the ability of chlorophyll to hold back anaerobic bacterial growth. The addition of reducing agents such as cysteine or thioglycollate to the medium in sufficient amounts overcomes this action and again permits growth to take place. Thus, there appear to be not only qualitative but also quantitative factors to consider in attempting to interpret the almost regular improvement of the infectious picture in clinical cases where chlorophyll is applied topically. Whether some mechanism which interferes with bacterial respiration exists similar to that occurring in relation to the sulfa compounds in infection requires further investigation.

Whether the prompt tissue repair response to chlorophyll therapy may not be the chief *modus operandi* in its control of infection seems entirely probable. At all events, it can be said as a result of these bacteriologic studies that chlorophyll is not strictly bactericidal, but that it does exert a definite bacteriostatic and even a bactericidal effect under suitable environmental conditions. When time and opportunity permit, these problems should be pursued to their ultimate solution, as the place of chlorophyll in the entire biologic pattern is so universally important that a thorough understanding of its mechanism is most essential.

**Summary.** 1. A brief résumé of our current concepts regarding the composition and chemical behavior of chlorophyll is presented.

2. Experimental evidence is introduced to demonstrate the total absence of toxic effects of the water-soluble derivatives of chlorophyll when administered, subcutaneously or intravenously, to animals or man.

3. Bacteriologic studies are reviewed which point towards an interference with the oxidation-reduction mechanism of bacterial respiration as explaining the rather striking bacteriostatic effects noted in the use of water-soluble chlorophyll derivatives clinically, and experimentally in animals, in the treatment of infected surface wounds.

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## A TUMOR OCCURRING IN THE SUPERIOR PULMONARY SULCUS

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SINCE the publication of a paper, "Superior Pulmonary Sulcus Tumor," by Dr. Henry K. Pancoast in 1932,<sup>16</sup> considerable controversy has arisen as to the origin and classification of the tumors which he described. Several writers<sup>1,3,5,9,13,15</sup> have expressed the opinion that the entire syndrome described by Pancoast could be produced by any inflammatory process or tumor mass arising within the lung or in the pulmonary sulcus. Some authors<sup>10,14,15,18</sup> have suggested that most tumors of this nature are bronchogenic or pulmonary in origin. Other authors<sup>7,8,16,17</sup> have attempted to present evidence to show that the tumors which they described arose outside the lung and pleura in the superior pulmonary sulcus, thus supporting the original contention of Pancoast. Dr. Joseph McFarland<sup>12</sup> has suggested a possible origin in the precervical sinus for the latter group.

Hall,<sup>9</sup> in a thorough review of the literature, has suggested that in most of the reported cases the lesion is an apical bronchogenic carcinoma with secondary involvement of the brachial plexus and the cervical sympathetic chain. Sympatheticoblastomas,<sup>6,11</sup> dural epithelioma of cord<sup>14</sup> squamous cell epithelioma<sup>5</sup> and metastatic carcinoma<sup>9,18</sup> have all been reported as producing an anamnesis and clinical course similar to the pulmonary sulcus syndrome.

The following case report and postmortem study is considered worthy of publication because it illustrates a superior pulmonary sulcus tumor in which the extrapulmonary origin of the tumor can be established, thus favoring the original contention of Pancoast that a primary tumor may arise in the pulmonary sulcus and that it may produce a definite clinical syndrome.

**Case Report.** B. R., white, male, age 47, American, electrician, was admitted to the hospital on Dec. 4, 1942, complaining of pain in his right scapular area and cough. He was in his usual good health until 2 months before admission, when he first noted a sharp piercing pain in his back medial to the right scapula. This pain soon became more constant, boring and aching in nature, and seemed to travel down the right arm. The arm then became numb and weak. Since the onset of this pain, he had a morning cough pro-

ductive of a moderate amount of yellowish white mucus and noted dyspnea on exertion. In the 2-month period prior to hospitalization he had lost 20 pounds in weight and complained of marked anorexia and weakness. Afternoon and night sweats were present, but never any hemoptysis. The systemic review, medical, social and family histories were entirely irrelevant.

*Physical examination* revealed a thin, pale, middle-aged, white man in apparent distress. His weight was 122 pounds and his sensorium was clear. A typical Claude Bernard-Horner's syndrome was present on the right. The right arm showed a moderate amount of muscular atrophy and a definite decrease in muscle power. Pain sensation was diminished in the right hand and forearm. The biceps, triceps, radial and ulnar reflexes were exaggerated on the right. Examination of the chest revealed dullness to percussion at the right apex and a diminution in breath sounds in that area. A definite tumor

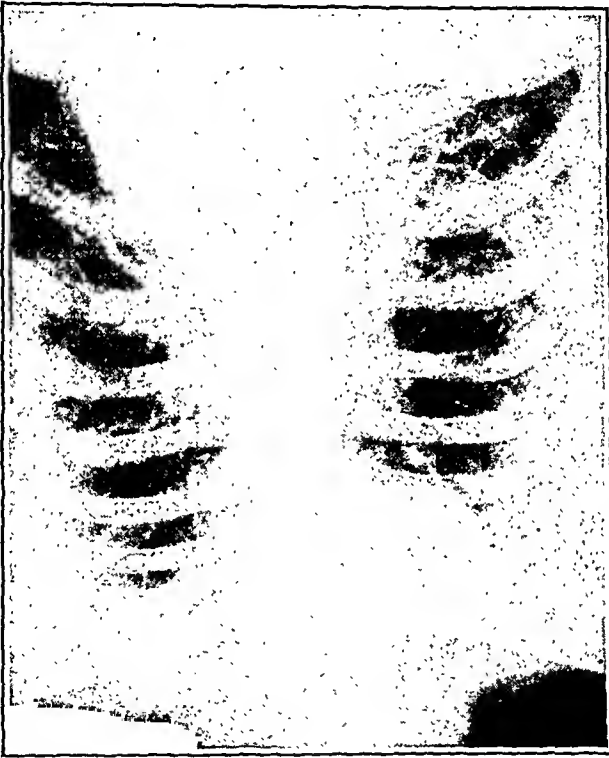


FIG. 1.—Chest demonstrating definite growth in the right apex.

mass was palpated in the suprascapular area on the right. The heart size was within normal limits, and the rate, rhythm and sounds were normal. Blood pressure was 104 over 44 in both arms with only slight variation on several readings. Examination of the abdomen revealed nothing abnormal. Sputum examination was repeatedly negative for acid-fast bacillus and blood. The blood Wassermann reaction was negative. Urinalysis was negative. Erythrocytes, 3,320,000; hemoglobin, 63%; leukocytes, 15,200, with 79% neutrophils, 20% lymphocytes and 1% eosinophils.

The *Koenigsmann examination* (Fig. 1) showed a lesion involving the apex of the right upper thorax. The lesion was indicated by a sharply defined, rounded, soft tissue shadow approximately the size of a tennis ball. This extended as low as the second rib anteriorly and filled the apex of the thorax. It displaced the esophagus slightly to the left but did not produce any evidence of erosion of the ribs or vertebrae.

When the patient was presented to the hospital tumor conference, the differential diagnosis of primary and metastatic malignancy was discussed, but no definite conclusions were reached. The radiologist suggested that the lesion was a primary growth possibly originating in the smaller bronchi of the right upper lobe. Operation was suggested as the treatment of choice for the purpose of biopsy and, if possible, lobectomy. After transfer to the Hamburg Tuberculosis Sanitarium for lobectomy further radiographic examinations were made of the chest. These showed an increase in the size of the tumor mass, but no bone erosion could be ascertained. A bronchoscopic study revealed a normal tracheobronchial tree with moderate compression of the trachea by an external lesion. On January 4, 1943, occult blood was found in the sputum and feces. Radiographic examination of the intestinal tract showed no definite lesion. About this time a walnut-sized mass appeared over the first interspace at the left sternal edge. Biopsy from this mass by Dr. A. Judd was reported by Dr. E. D. Funk as probable metastatic carcinoma of squamous cell origin.

With the general picture of rapid deterioration, erosion of the sternum, and rapidity of growth of the pulmonary lesion, a lobectomy was considered foolhardy. The patient was returned to the Reading Hospital on Jan. 14, 1943, in a cachectic state and expired shortly thereafter. Just previous to his demise on Jan. 22, 1943, two soft tissue nodules were noted in the upper abdominal wall.

**Necropsy Findings.** *Macroscopic.* Superior pulmonary sulcus tumor with erosion of the manubrium, first and second ribs, first and second thoracic vertebra, and involvement of the brachial plexus and sympathetic ganglion; metastasis of tumor to skin, peritoneum and right and left adrenal medulla; early bronchopneumonia of right lower lobe; inanition; pathologic fracture of first rib on right side; pulmonary emphysema; chronic passive congestion of liver and spleen.

The body was that of an extremely emaciated white male, weighing approximately 100 pounds. The skin had a sallow, yellow color. The sclera was icteric, the right palpebrum was closed and the pupils were round and equal. Above the right clavicle was a protruding apical mass. The chest was emphysematous. Just lateral and left to the manubrium was a nodular growth measuring  $4 \times 5 \times 1.5$  cm. with a healed incision over its surface measuring 4 inches in length. The abdomen was scaphoid in type. Below the rib margin in the right and left mid-clavicular line were two hard nodular masses. Another mass over the right hip was also noted.

When the peritoneum was opened a large cauliflower mass measuring  $5 \times 5 \times 3$  cm. was found anteriorly in the abdominal wall just above and lateral to the umbilicus. No free fluid was found. The intestinal tract was collapsed and normal throughout. The right anterior abdominal wall contained a nodular mass beneath the peritoneum which measured 3 cm. in diameter. These growths were friable, necrotic and yellow in color.

The right pleural cavity contained a tumor mass lying in the extreme apical portion. It was fungating, friable, necrotic, yellowish white in



The apical portion of the upper lobe was attached to the mass by adhesions which were readily broken. The mass extended anteriorly to the level of the second rib and posteriorly to the fourth interspace. It had eroded through the first and second ribs, causing a pathologic fracture of the first rib, and also into the first and second thoracic vertebrae. It extended superiorly into the brachial plexus to the level of the cricoid cartilage. The right lung measured 23 x 22 x 5 cm. and weighed 610 gm. At the apex there was a scarred area measuring 8 x 8 cm. which was attached to the tumor mass. In this area the lung parenchyma was compressed and no evidence of tumor could be found on gross section. Several small, patchy, yellowish red areas were present at the base. The bronchioles were filled with a yellowish exudate.



FIG. 2.—Sulcus tumor showing cellular degeneration and round cell infiltration. ( $\times 150$ .)

The right adrenal gland measured 8 x 6 x 3 cm., and the medulla was replaced by a large, fungating, yellowish white mass of necrotic tumor tissue. Only a narrow strip of yellowish orange cortical substance remained. The left adrenal measured 6 x 5 x 3.5 cm. and showed the medulla replaced by a growth similar in gross appearance to that in the medulla of the right adrenal. The left and right kidneys presented a flattened area in the superior pole where the suprarenal glands had rested. They were entirely free of the adrenals and showed no evidence of any tumor growths. The cortex and medulla were well differentiated and the capsule striped with ease. The testes, epididymis and prostate gland were all small and normal in appearance.

*Microscopic.* Study of the sulcus tumor mass revealed extensive cellular necrosis in which were found islands and masses of tumor cells (Fig. 2). The cellular portion of the tumor was composed of irregularly shaped large cells with a slightly granular or clear cytoplasm and a large round or oval vesiculated nucleus with a prominent nucleolus. Some nuclei, however, were pyknotic and hyperchromatic. No tendency toward any definite acinar formation of the tumor cells was noted (Fig. 3). Throughout these masses of tumor cells, lymphocytes were diffusely scattered. In the center of a number of islands of these immature squamous epithelial cells was an area of cellular necrosis

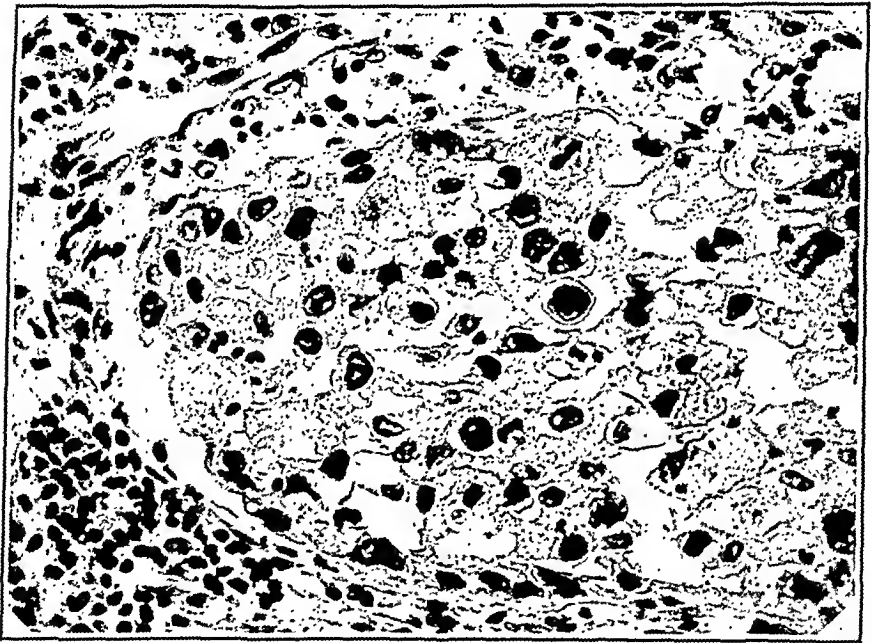


Fig. 3.—Neoplastic area showing mitosis and anaplasia. ( $\times 640$ .)

which was surrounded by round cells. Mitotic figures were frequent to the tumor, revealed an infiltration of round cells, tumor cells, and a partial atelectasis of the alveoli. Several bronchioles were present. These showed no evidence of any malignant degeneration. The lung tissue of the right lower lobe revealed a patchy bronchopneumonia. Here the alveoli were filled with polymorphonuclear leukocytes and monocytes. The adrenal glands revealed a partial pressure atrophy of the cortical portion. The capsule was intact. All 3 zones were narrowed. The entire adrenal medulla was replaced by exactly the same type of tumor cells which were present in the sulcus tumor. Necrosis as well as lymphocytic infiltration was in evidence (Fig. 4). The peritoneal implants also revealed the exact type of tumor found in the sulcus mass. The kidneys revealed no evidence of tumor growth. The glomeruli and their Bowman's capsules were entirely without pathologic change. The tubules showed a slight amount of cloudy swelling.

**Discussion.** The clinical syndrome described by Pancoast included (1) pain in the shoulder region often radiating down the arm and eventually into the fingers, (2) Horner's syndrome, (3) local destruction of the first 2 or 3 ribs, and (4) atrophy of the muscles of the hand. The Roentgen observations were described as a small, sharply defined, soft tissue shadow in the apex of the thorax which usually appeared insignificant when compared with the symptoms of the patient. Accompanying this is a destruction of one or all of the upper 3 ribs in



Fig. 4.—Tumor cells replacing adrenal medulla and compressing adrenal cortex. (X 150.)

their posterior aspects and the adjacent transverse processes and sometimes slight vertebral erosion.<sup>16</sup> An important feature in most of the reported cases has been the absence of signs or symptoms of pulmonary disease. Most of the tumors have occurred in men and, regardless of their origin, the life expectancy is 1 year or less.<sup>15</sup> The syndrome described by Pancoast was present in this case. The clinical course from the onset of pain to death was extremely rapid, totaling only 3½ months. Physical signs and symptoms of a pulmonary lesion which usually have been strikingly absent in the reported cases were present in this case, apparently due to the large size of the

The rapid growth exhibited by this lesion by Roentgen examination is also somewhat unusual. It is also worthy of note that none of the Roentgen examinations demonstrated evidence of osseous erosion. The tumor mass in the pulmonary sulcus at postmortem examination was undoubtedly extrapulmonary and was so proven by microscopic sections. Dr. McFarland, who made a diagnosis of spino-cellular carcinoma in one of Pancoast's cases, reviewed the histologic sections of this patient. He expressed the opinion that this tumor was composed of cells which are squamous epithelial cells but did not reach the prickly stage and that the adrenal medullary tumors and the sulcus tumor were the same cell type. The unusual amount of necrosis in the tumor is evidence of its rapid growth. This feature had also been noted by Barton<sup>1</sup> in his case. The finding of a similar type of tumor cell in both the adrenal medullas, as did Jacox,<sup>10</sup> raises the question of a possible adrenal medullary or sympathetic ganglion origin. Was this tumor primary in the pulmonary sulcus or in the adrenal? From the history obtained, it is not possible to state whether the Horner's syndrome preceded the onset of scapular pain or whether it followed the onset of pain. This thought is projected with the idea that perhaps those tumors which are not bronchogenic carcinomas do arise in the precevicul sinus or the cervical sympathetic ganglion. Therefore, the primary origin of this tumor cannot be definitely established and Pancoast's theory of an origin from the precevicul sinus deserves careful consideration.

**Summary.** 1. A case is reported which illustrates many of the features originally described as representing the superior pulmonary sulcus tumor.

2. Evidence is presented to indicate an extrapulmonary origin for this tumor.

3. Controversial evidence regarding the nature and origin of these tumors is briefly discussed.

I am indebted to Drs. Erwin D. Funk, George W. Chamberlin and Howard U. Miller for permission to publish the case presented and for their assistance in the preparation of this paper.

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# PROGRESS

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### PENICILLIN

#### A REVIEW OF THE LITERATURE THROUGH 1943\*†‡

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Factors inhibiting bacteria have been known for almost 50 years (Emmerich and Low, 1899),<sup>45</sup> (Emmerich and Saidah, 1900).<sup>46</sup> The considerable literature that has arisen concerning the antagonistic relations of microorganisms has been completely and adequately reviewed by Waksman (1941).<sup>124</sup> and Waksman and Woodruff (1942).<sup>125</sup> However, only during the last several years has the general interest of the profession in the subject—primarily due to the advent of penicillin—been aroused. While the span of life of penicillin has been short, over 100 articles dealing with it have been published. Some of these are in journals inaccessible except in the larger libraries. Accordingly, the purpose of this paper is to collect and summarize the knowledge of penicillin up to the present time. Unless some articles have been overlooked unintentionally, the present bibliography is complete to January 1, 1944.

**Historical.** Fleming (1929)<sup>48</sup> observed that the growth of certain bacteria was inhibited by the chance introduction of a mold into culture plates containing bacteria. Because the genus to which this species of mold belonged was so widespread in all sorts of decaying matter, such a discovery was not remarkable. The mold, tentatively identified as *Penicillium rubrum*, was a member of the great company that rotted fruit, vegetables, meat and grain, and invalidated many kinds of biologic experiments. Such organisms were so conspicuous in all sorts of stale decaying matter that they constituted the common conception of "mold."

\* Written as part of graduate work at Stanford Univ. Medical School and was made possible by the facilities of the Lane Medical Library. Dr. A. L. Bloomfield gave suggestions and advice.

† The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

‡ This article is appearing in briefer form in the U. S. Naval Bulletin for the use of men overseas.

The year following Fleming's discovery, Thom (1930)<sup>119</sup> in a comprehensive monograph stated that outside of the ripening effect upon Camembert and Roquefort cheeses, the possibilities of usefulness of the *Penicillium*—a group of hyphomycetes which produced conidial fructifications in the form of a brush or broom (*P. Penicillium*, *penicillium*, pencil, so named in allusion to the tufts at the ends of the conidiophores)—remained unknown. The utilization of such an agent seemed to be confined to the laboratory, as Fleming (1930)<sup>120</sup> (1932)<sup>121</sup> reiterated the fact that penicillin had remarkable antibacterial properties and was useful in isolating bacteria from another. At the same time, he, as well as Clutterbuck (1932)<sup>122</sup> identified the mold as *Penicillium notatum* Westling. Later, Reid (1935)<sup>123</sup> investigated a large number of hyphomycetes, and found that only Fleming's mold had any antibacterial effect.

Here the matter rested until Florey, Abraham, Chain, and others (1940)<sup>124</sup> (1941)<sup>125</sup>, perhaps stimulated by the work of Dubos (1931-1940),<sup>126-128</sup> published comprehensive reports of their investigations of penicillin. These reports were so enthusiastic that large numbers of investigators have since been stimulated to action in this field.

**The Media.** Fleming<sup>129,130</sup> stated that the mold grew in a variety of different media, but best in tryptic digest broth. Later, Abraham<sup>131</sup> recommended Clutterbuck's<sup>132</sup> modification of the synthetic Czapek-Dox media:

Sodium nitrate (NaNO <sub>3</sub> )	3.00
Potassium acid phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1.00
Potassium chloride (KCl)	0.50
Magnesium sulfate (MgSO <sub>4</sub> ·H <sub>2</sub> O)	0.50
Ferrous sulfate (FeSO <sub>4</sub> ·H <sub>2</sub> O)	0.01
Glucose	40.00
Distilled water, q. s.	1000 cc.

This formula was modified by McKee and Rake<sup>133</sup> who substituted brown sugar for glucose. Hobby *et al.*<sup>134</sup> reduced the amount of brown sugar to 20 gm. and made other slight changes. Foster, Woodruff and McDaniel<sup>135</sup> stated that formula containing brown sugar gave the best results, but suggested doubling the amount of sodium nitrate. They made a great number of modifications in the basal media, but failed to find any noteworthy improvement. However, ferrous sulfate and potassium chloride could be eliminated entirely, and the quantity of potassium and phosphate lowered considerably without reducing the penicillin-productive power of the mold. Kocholaty<sup>136</sup> substituted manganese for iron, and others<sup>137</sup> stated that the addition of zinc was helpful. The use of lactose (5%) was effectual in increasing antibacterial titer for some but not for others.<sup>138</sup> Challinor buffered the media more by the addition of mono- and di-hydrogen phosphates.<sup>139</sup>

Lately, Taylor<sup>140</sup> compared the growth of *P. notatum* upon Czapek-Dox synthetic medium, Difco stock heart infusion broth, and Amino (a trade name for a product consisting of an enzyme digest of purified casein and pork pancreas in which the proteins were hydrolyzed to 75% di- and tri-peptides). According to him, the growth was far better on Amino than on any other substrate.

**Growth.** Only known identified strains of the highest potency were selected, and these were rigidly controlled to prevent degeneration.<sup>141,142</sup> The number of vegetative transfers was reduced to a minimum. A convenient method of keeping cultures at a high level was by allowing sporulation of the mold, mixing the spores with dry sterile sand, and drying

the mixture at low temperature as needed. Spores from this master race were sown upon suitable media. Frequently, the mold was grown first upon a slope of Sabouraud's media, from which a spore suspension was made by shaking with sterile water, and implanting upon sterile media. After inoculation, the shallow medium (1.5 to 2 cc.) was not disturbed so that the growth did not conglomerate into balls. A delicate fluffy growth appeared 24 hours later on the bottom of the vessel, reached the top of the solution on the 3rd day, and soon the whole surface was covered with a dry mycelium which began to turn green almost immediately. By the end of a week, the growth consisted of a compact continuous wrinkled dark green felt whose upper surface containing yellow spores could not be wetted with water. Later, the mold became faded and gray. The substrate gradually turned yellow during this period of growth from a pigment, chrysoengin, which had no antibacterial action.<sup>22</sup> However, penicillin of high assay value was rarely found in the absence of this pigment.<sup>58a</sup>

The mold flourished at 22° C. (range 20° to 26°), but failed to grow at 37° C. Growth occurred over a wide range of oxygen tensions, but not anaerobically.<sup>2</sup> The production of penicillin was, in part, dependent upon the reaction of the media.<sup>2,58a,117</sup> The reaction began at the neutral point, reached pH 3 at the end of the 3rd day, gradually rose to pH 5 to 6, and finally, as the color of the mycelium faded, became alkaline. The production of penicillin was maximum slightly below the neutral point. Yield. Usually the yield was 2 to 4 (rarely 6 to 8) Florey units per cc. of culture fluid.<sup>2</sup> As noted above, changes in the culture media made some difference in the amount of penicillin. Certain strains produced more penicillin than others.<sup>58a,65,88</sup> Challinor<sup>20</sup> decreased the time required for maximum production from 10 to 15 days<sup>22</sup> down to 5 to 6 days by the addition of 0.1 to 1 cc. of media from a previous batch to 200 cc. of the current substrate. McKee and Rake<sup>88</sup> found that while highly active filtrates were obtained in 10 days after original inoculation, rethoeding the used fungus with fresh media caused a maximum concentration of penicillin within 6 to 7 days. There was no relation between the size of the thallus and the antibacterial titer.<sup>117</sup>

*Parasites on the Mold.* Dobbs,<sup>39</sup> in emphasizing the importance of keeping the culture media sterile, stated that Piptocephalus was parasitic on *P. notatum*.

*Large-scale Production.* While the method of growing the mold in shallow media described above is of historical interest, almost all manufacturers now use a modification of the technique described by Clifton.<sup>21a</sup> He constructed a tube containing a column of wood shavings 4½ feet long. The top of the apparatus contained two holes, one for the passage of culture media (Czapek-Dox containing 4% glucose and 0.1% Difco yeast extract) and the other for the admission of a stream of air. After the column was sterilized by steam, the shavings were wet with culture media, and *P. notatum* was introduced. The medium was allowed to flow at the rate of 600 to 800 ml. daily. While the prevention of bacterial contamination was a problem, it was not insurmountable, and it was believed that this method offered the best possibility for the production of penicillin in large quantities.

*Penicillin. Extraction and Purification.* Penicillin was obtained primarily from the substrate, but a small amount was found in the thallus.<sup>118</sup> The most detailed account of the production of penicillin was recorded by Abraham, Chain, Florey and others,<sup>2,5</sup> as follows: The filtered, chilled

(40° C.) culture fluid was acidified with 10% phosphoric acid to pH 1.9, and extracted in lots of 2 liters with an equal volume of amyl acetate. The solution (in 2 liter lots) was mixed with 400 cc. of water, N/30 baryta water gradually was added to bring the pH to 6.2. The resultant deeply-pigmented red-brown solution was filtered, and treated with animal charcoal. The mixture was filtered, chilled to 4° C., and one-third volume of cold ether was added. The watery layer, acidified to pH 1.9 with 10% phosphoric acid, was extracted with vigorous shaking. Three extractions done in this manner were followed by passage through an alumina column 50 cm. long, with the formation of the following chromatogram:

- (1) Dark brown layer—containing small amounts of penicillin.
- (2) Light yellow layer—containing bulk of penicillin.
- (3) Orange or lemon yellow layer—containing small amounts of penicillin.
- (4) Purple layer—containing no penicillin.

The second fraction separated mechanically from the others was eluted 4 times with M/15 phosphate buffer (pH 7). The combined yellow eluates were extracted thrice with ether at pH 2. From this, the penicillin was returned to aqueous solution by adjustment to pH 6 with N/30 baryta water. The inactive pigment remained in the ether layer. The active substance was again extracted with ether, and the solution was passed through another alumina column. The same layers were obtained but only the second fraction was used. The process of purification was repeated as above.

The next step was reduction with aluminum amalgam, following which the solution was cooled to 4° C. and extracted with amyl acetate. The amyl acetate was then extracted with water at pH 7. The aqueous solution was reextracted at pH 2 at 5° C., and passed through an alumina column. This time only 3 fractions were obtained:

- (1) Very light brown.
- (2) Almost colorless.
- (3) Greenish yellow.

Division of the column was facilitated by observation in ultra-violet light, because the first and third fractions were fluorescent. The penicillin was eluted from the second fraction with phosphate buffer. After 2 further chromatographic adsorptions from amyl acetate, the resulting column was almost colorless and homogeneous. Elution with phosphate buffer, extraction with amyl acetate, and reextraction with N/30 baryta water at pH 5 yielded on lyophilic drying a faintly yellow baryta salt of penicillin with an activity of 500 units per mg. Dawson, Meyer and others<sup>28,90</sup> adjusted the culture media to pH 3-4, saturated it with ammonium sulfate, and extracted the product with chloroform. The active agent was removed by phosphate buffer at pH 7.2. Extraction with chloroform and buffer was repeated, and less acidic pigments separated from most active fraction by chloroform extraction at different acidities. The penicillin was obtained as a free acid by precipitation from petroleum ether or as ammonium salt by saturation of a chloroform-benzol solution with dry ammonia gas. According to Catch, Cook and others<sup>18,23</sup> the most satisfactory method was by chromatographing from an organic solvent upon a column consisting of a water-retentive support (silica gel) associated with an organic base such as a hydroxide or carbonate of alkali or alkaline earth metal. The latter was applied either in the form of a dilute solution in water or was precipitated on a carrier before use. The penicillin



was extracted primarily on the relative strengths of the component acids of the media.

# Properties.

Penicillin has been obtained in the form of a free acid and the salts of barium, potassium, sodium, ammonium calcium, silver and strontium.<sup>2,3,5,8,28,54,55,70a,90</sup> Penicillin is partially soluble in ether at pH 7.2, completely soluble at pH 2, but insoluble in alkaline solutions.<sup>22,48</sup> It is freely soluble in water, weak saline solution, alcohol, ethyl acetate and dioxan. Penicillin is less soluble in benzene, chloroform and carbon tetrachloride. The salts are hygroscopic, the barium salt less so than the others. All rapidly lose their activity on exposure to air. The strongly dibasic acid is extremely unstable, probably from the lability of free carboxyl groups.<sup>5</sup> Penicillin, in the form of the acid or its salts, is easily oxidized by potassium permanganate and hydrogen peroxide, but is less sensitive to reducing agents. It is unstable toward dilute acids, alkalis, various heavy metal ions<sup>6</sup> and moist organic bases such as aniline, methyl aniline and dimethyl aniline. Penicillin may be treated for 1 hour at 56° C. or boiled for a few minutes without loss of activity; autoclaving at 115° C. for 20 minutes completely destroys it.<sup>48</sup> Penicillin does not reduce Fehling's solution, but the blue color changes to green from formation of complexes. The barium salt is strongly dextro-rotary,<sup>6</sup> but the strontium salt is optically inactive.<sup>18</sup>

The empirical formula has been suggested by Abraham and Chain,<sup>4,5</sup> who analyzed the dried barium salt with the following results: C—44.3, H—4.85, N—4.13, C—Me 11.6, Ba—22, no phosphorus, sulfur, or O Me; no easily reducible double bonds. From this, they suggested the formula of  $C_{23}H_{30}O_{10}N_2Ba(M.W. 640-645)$  or  $C_{23}H_{30}O_9N_2$ . A formula containing  $C_{25}$  was not excluded. These formulas agree essentially with that ventured by Catch, Cook and Hellbron.<sup>18</sup>  $C_{23}H_{33}O_{11}NSr$ . Some steps have been taken towards establishing the structural formula by Holdiday.<sup>75</sup> From spectrographic examination (maxima at 247 and 300 mμ), he suggested the possible characteristics: polysubstituted hydroaromatic ring; acidic groups, probably carbonyl; and possibly the presence of a trisubstituted αβ unsaturated ketone group.

The molecule has been ruptured by Abraham<sup>4</sup> and Catch.<sup>18</sup> Apparently, the following substances were formed:

- (1) Carbon dioxide.
- (2) Colorless water-soluble acid, which on further hydrolysis gives rise to a simple peptide.
- (3) Yellow insoluble pigment ( $C_{16}H_{20}O_6$  or  $C_{16}H_{18}O_5H_2O$ ).
- (4) Acetaldehyde.

# Assay.

A completely satisfactory method of assay is not available at the present time. A need for the establishment of a suitable method is pressing, particularly for the determination of antibacterial titer of penicillin in the substrate of the culture. Foster<sup>27</sup> formulated the requirements of a suitable assay: no pretreatment, accuracy, results available shortly, readings unequivocal, and the demonstration that only one penicillin is present.

The various methods may be classified under one of the following basic principles:

1. *Oxford Cup*. The progenitor of this method was that of the hole or agar cup technique described by Fleming.<sup>48,52</sup> Florey *et al.* modified the method by implanting tiny glass or porcelain cylinders containing penicillin in nutrient plates heavily seeded with *Staphylococcus aureus*. The

plates were incubated for 12 to 16 hours at 37° C., and the surrounding zone of bacterial inhibition read. A standard solution produced an average zone of inhibition of 24 mm., which was defined as a Florey (Oxford) unit. While keeping the basic technique, Foster and Woodruff<sup>166a</sup> introduced an important modification. They stated that since attempts to stabilize cultures of *S. aureus* were fraught with difficulty, a sensitive strain of *Bacillus subtilis* was substituted. They found that not only were there larger zones of inhibition, reducing the percentage deviation of error in measuring them, but also the zone edges were knife sharp, with the growth of organisms standing in clear contrast to the zone of inhibition. Recently, Dowdy and the Vincents<sup>167</sup> substituted paper disks saturated with penicillin for the cylinders.

2. *Serial Dilution.* This was done in tubes containing nutrient broth or upon agar plates. The essential points of the plate method were as follows: various amounts of the sample were mixed with 10 cc. melted agar, which after solidification, was streaked with a test organism. The plates were incubated overnight, and the concentration which inhibited the growth was read.

A popular technique was titration in liquid broth. Fleming<sup>2</sup> used a broth consisting of peptone (1 gm.), sodium chloride (0.5 gm.), glucose (1 gm.), Andrade's indicator (1 cc.) and water (q. s. to 100 cc.). A 3 mm. loop from a 24 hour broth culture of *S. aureus* was inoculated into 5 cc. of the media. Serial dilutions of penicillin were made, and added to the broth. After incubation for 24 hours, the end-point was the appearance of a red color in the media. From a comparison between a known standard and the unknown, the latter was calculated.

Rammelkamp<sup>97</sup> tested the susceptibility of a number of organisms to the action of penicillin. Hemolytic streptococci were found to be 4 to 16 times more sensitive to penicillin than staphylococci, and hence these organisms were to be preferred. The test organism was a 12 hour broth culture of a Group A strain of hemolytic streptococci, which was inoculated into veal infusion broth containing 1% erythrocytes. This was diluted so that the final number of organisms varied between 1000 and 10,000 per cc. Two series of tubes were set up: one for the unknown and the other for the control. The latter contained 20 Florey units per cc. of 0.85% normal saline solution. Serial dilutions were made in the accepted manner, incubated for 18 hours, and the tubes were then examined for hemolysis. The end-point was usually clear, but as a check for sterility, the contents of the tubes on both sides of this point were streaked upon a blood agar plate. Calculations made from comparison of the end-points of the unknown and control gave the concentration of the drug in the unknown solution.

Hobby, Meyer and Chaffee<sup>22</sup> modified the method slightly by using a standard strain of hemolytic streptococcus (C 203 M). Their end-point was the failure of a given dilution of penicillin, culture media and erythrocytes to develop turbidity after 24 hours. They reported the activity of penicillin as the number of micograms necessary to inhibit the growth of 2 to 4 million hemolytic streptococci. Recently, Wilson<sup>126</sup> described a test based upon the inhibition of hemolytic streptococci using 5% washed sheep cell suspension as an indicator. She stated the results could be read within 4 hours.

Since the Florey (Oxford) unit as defined above did not apply to the serial dilution method, Florey and Jennings<sup>54</sup> defined this unit in terms of the latter as follows: A unit is that amount of penicillin which when dis-

solved in 50 cc. of meat extract broth completely inhibits the growth of a test strain of *S. aureus*. Expressed differently, a meat extract broth containing 1 unit of penicillin per mg. just inhibits the growth of *S. aureus* in dilutions of 1 to 50,000.

3. *Turbidimetric*. Foster *et al.*<sup>56, 57, 58</sup> stated that the inhibition of growth of a culture of *S. aureus* in liquid media was a function of the penicillin concentration, and that the turbidity of the mixture as measured by a colorimeter was in inverse ratio to the strength of the penicillin. Recently, the technique was modified by the substitution of *B. anthracis* for *S. aureus*. The time required for an end-point was reduced to 3 to 5 hours. This method was peculiarly suitable for use in assaying substrates.

4. *Other Methods*. Foster and Wilker<sup>59</sup> described assays with which they had little success:

- (1) Methylene blue reduction of washed cells of susceptible organisms.
- (2) Inhibition of luminescence in cultures of luminescent bacteria.
- (3) Microscopic observation of cessation of motility of bacteria.
- (4) Microscopic observation of the appearance of enlarged or involution cell shapes.

5. *Titration of Acid Formed by Lactic Acid Bacteria*. As noted above, the ideal method of assay has not been suggested. However, the easiest and most practicable for routine use is the technique of serially diluting the penicillin in tubes containing broth, test organism and erythrocytes. However, this as well as the other methods, has disadvantages, which Foster and Woodruff<sup>57</sup> have summarized:

- (1) *Oxford Cup*. (a) Affected by the pH of the media. (b) Organism and penicillin must be inoculated within a very short time of one another.
- (2) *Serial Dilution (Liquid Broth)*. (a) Sterile samples are necessary or else sterility is effected by passage through a Seltz filter. (b) End-point may not be definite.
- (3) *Serial Dilution (Plate Method)*. (a) Affected by changes in pH. (b) Presence of bacteria containing penicillinase may affect end-point.
- (4) *Turbidimetric*. (a) Large quantity of sample necessary. (b) Too difficult for routine use.

**Relatives of Penicillin.** In the literature one finds the names of related and degradation products of penicillin. It is impossible to predict which, if any, are identical, which will be discarded, or what new substances will be added subsequently.

*Penicillin B*. Roberts,<sup>108</sup> Van Bruggen<sup>123</sup> and others isolated a substance which they called penicillin B to differentiate it from the original penicillin of Abraham. The first report stated that this compound was readily separated from the culture media by adsorption upon benzoic acid, and purified by repeatedly dissolving in acetone. Subsequent reports stated that penicillin B was better extracted by precipitation with uranium acetate, centrifuging and siphoning off the supernatant fluid. The latter was washed with water, and extracted with a volume of 0.2 M phosphate buffer sufficient to give the mixture a creamy consistency. After standing for several hours, the mixture was centrifuged, reextracted, and finally salted out with ammonium sulfate. Penicillin B occurred as a light yellow readily-soluble powder, which gave positive tests for protein. Study suggested that it was a flavo-protein of enzyme nature. It was lethal to both Gram-negative and positive organisms, but the presence of glucose

was necessary for its antibacterial action. The glucose was oxidized to  $R \cdot CHO + H_2O + O_2 \rightarrow R \cdot COOH + H_2O_2$  with the formation of hydrogen peroxide, to which antibacterial action was attributed. Unfortunately, penicillin B was toxic to mice, although non-cumulative; attempts to detoxify it were unsuccessful.

*Penatin*. Kocholaty<sup>79,80,81</sup> stated that certain strains of penicillin differed not only in the rate of production of penicillin, but also in the compounds produced. He isolated a substance called penatin, and prepared it by adsorption with kaolin at pH 4, elution of the washed kaolin with pyridine or sodium phosphate at pH 6.3, followed by precipitation with dioxane. The product was dissolved in water, then dried by the lyophilic method. Penatin was a yellowish hygroscopic powder completely soluble in water. While it was sensitive to alkalis, it was more resistant to acids. Penatin far surpassed penicillin not only because it was bacteriostatic in dilutions of 1 to 400 millions, but also because it was antagonistic to certain Gram-negative organisms (*E. coli* and *Brucella abortus*).

*Notatin*. The co-workers of Coulthard<sup>82</sup> isolated a substance prepared by concentrating the culture filtrate to one-fifth its volume under mild conditions, and precipitating the concentrate with acetone or tannic acid. In the latter case notatin was extracted from tannic acid complexes formed. Regeneration of the crude substance was effected from complexes formed with tannic acid or Reinecke's salt. Notatin was a buff-colored powder freely soluble in water, but insoluble (6.7%), nitrogen (11.8%) and phosphorus (0.58%). It exhibits many of the reactions of protein, and has a molecular weight of 73,000. Since it has absorption spectrum maxima of 280, 375 and 465 m $\mu$ , it is classified as a yellow enzyme. Notatin, an aërodehydrogenase, executes its activity only under certain well-defined conditions: presence of oxygen and glucose and the absence of appreciable amounts of catalase. Its antibacterial activity may be due to the liberation of hydrogen peroxide.<sup>84</sup> The best preparations completely inhibit the growth of *S. aureus* in dilutions of 1 to 1 billion, and it is active against *Streptococcus hemolyticus*, *Diplococcus pneumoniae*, *Escherichia typhosus* and paratyphosus, *Vibrio cholera*, *Bacillus anthracis* and *B. proteus vulgaris*.

*Comparison of Penicillin B, Penatin and Notatin*. Birkinshaw and Rastriek,<sup>84</sup> Foster<sup>85</sup> and others pointed out that these 3 substances were probably identical, and that their formation was dependent upon the pH of the media. Normally, using materials inherently impure containing traces of heavy metals (zinc, copper, manganese) or organic supplements (yeast extract), the pH underwent normal variations. However, extremely pure ingredients effected no secondary rise in the pH which remained at pH 3-4. It is at the latter ranges that the above substances are formed, and it accounts for the presence of notatin (penatin, penicillin B, coli factor of Waksman and Woodruff<sup>86</sup>) in many cultures of *P. notatum*. In a word, notatin is formed in acid media, while penicillin is produced in neutral media. It is possible that different strains vary in the production of each.<sup>58a</sup> Of the substances isolated, notatin seems to hold the most promise, for it adds a number of organisms to the growing list of those adversely affected by products of the growth of *P. notatum*.  
*Penicidin*. This was reported by Atkinson,<sup>10,11</sup> who noted that a certain penicillium (mold 9) produced a substance which was inhibitory to Gram-positive and negative organisms. The mold grew on a modified Czapek-

Dox media at 18° to 20° C. for 5 days, after which the active component was removed by concentrating the substrate *in vacuo* to one-tenth its original volume, filtering, adjusting to pH 7, and extracting with ether for several hours. The ether extract was washed with water, adjusted to pH 4, and the penicillin was removed by dilute acid. The reaction was changed to neutral, the material was reextracted with ether and evaporated *in vacuo* to a small volume. The addition of petroleum ether caused an immediate precipitate which on standing in the cold settled out as a pale yellow oily layer of incompletely purified penicillin. Penicillin was relatively heat-resistant; not readily destroyed by hydrochloric and sulfuric acids; easily inactivated by potassium and barium hydroxides; soluble in chloroform, benzene, ether, ethyl alcohol and dilute mineral acids; and insoluble in petroleum ether. Penicillin was active against *Librethella typhosa* in dilutions of 1 to 100,000.

*Penicillic acid.* Duffin and Smith<sup>37</sup> and Oxford, Raistrick and Smith<sup>38</sup> reported that highly active penicillin preparations kept at pH 2 exhibited a rise of optical activity, which, after reaching a maximum, remained constant. Following this phenomenon, only part of the penicillin previously extractable by ether could be obtained. The remaining substance, a constituent of the molecule of penicillin, was strongly dextro-rotatory

$\left(\frac{A}{B} + 600\right)$ . The acid, which when in solution had a pale blue fluorescence in ultra-violet light, crystallized from water in brilliant rhombo or hexagonal plates. It had some of the properties of amino acid, and it did not give a blue color with ferric chloride—a characteristic of penicillamine.

*Penicillamine.* This was obtained by Abraham, Chain *et al.*<sup>6</sup> by hydrolyzing barium penicillin at 100° C. for 1 hour by N/10 sulfuric acid. The base thus recovered was precipitated from a concentrated solution by bichloride of mercury. After demercurizing with hydrogen sulfide and evaporation at room temperature under diminished pressure, a mass of homogeneous crystals (penicillamine) was obtained. Penicillamine had the formula of  $C_6H_{11}O_4N \cdot HCl(C_6H_5O_6 + NH_3 - 2H_2O)$ , was optically inactive, and seemed to be an integral part of the penicillin molecule. The nitrogen was in the form of an amino acid group, although not typical of an alpha-amino acid. Among its other properties, it gave a deep blue color with ferric chloride, reduced ammoniacal silver nitrate on gentle warming, and produced a green color with Fehling's solution. The exact significance of this degradation product was unexplained.

*Esters.* Although Abraham and Chain<sup>6</sup> were unsuccessful in their attempts to esterify penicillin by the action of various alkyl iodides on the silver salt, this did not deter Meyer *et al.*<sup>9,22</sup> who successfully prepared methyl, ethyl, n-butyl and benzoyldiethyl esters by letting the free acid of penicillin react with the corresponding diazo compound. The ethyl and normal butyl esters appeared to be stable and were not destroyed by acids. Fleming<sup>18</sup> noted that penicillin acted best against pyogenic cocci and the diptheria group while Gram-negative bacilli were insusceptible. Susceptibility occurred in a 1% solution; or, if the organism were resistant, did not take place in a 10% solution. The first use of penicillin was a laboratory aid to prevent the growth of more robust organisms in a

mixed culture.<sup>26,51,53</sup> In fact Fleming (1932)<sup>49</sup> stated penicillin's main uses were:

- (1) To isolate hemophilic bacteria such as *B. pertussis* and *B. influenzae* from streptococci and pneumococci.
- (2) Isolation of one partially insensitive microbe in pure culture from a mixture of more sensitive organisms (acne bacillus from staphylococci).
- (3) Separation of Gram-negative cocci from other mouth organisms.
- (4) Demonstration of bacterial antagonisms.

The chief use, then, of penicillin was as a laboratory aid until the work of Abraham, Florey *et al.*<sup>2</sup> resulted in the impetus necessary to use the drug in man. Since then, lists of organisms against which penicillin was found to be active have been published by several authors;<sup>2,48,88</sup> but the list usually referred to is that of Dawson *et al.*<sup>28</sup> and Hobby, Meyer and Chaffee,<sup>72</sup> which summarized the susceptible and resistant organisms:

Susceptible	Insusceptible
Pneumococcus	<i>Hemophilus influenzae</i>
<i>Strep. hemolyticus</i>	<i>B. coli</i>
Staphylococcus	<i>B. typhosus</i>
Meningococcus	<i>B. dysenteriae</i>
Gonococcus	<i>B. proteus</i>
<i>Strep. viridans</i>	<i>B. paratyphosus A</i>
<i>B. subtilis</i>	<i>B. enteritidis</i>
<i>Clostridium welchii</i>	<i>B. pyocyaneus</i>
<i>Vibrio septique</i>	<i>B. fluorescens</i>
<i>Clostridium histolyticus</i>	<i>B. prodigiosus</i>
<i>B. sporogenes</i>	Friedländer's bacillus
<i>B. edematis</i>	<i>S. albus</i> —1 strain
<i>B. sordelli</i>	<i>Micrococcus albus</i> —1 strain
Lactobacillus	<i>Monilia albicans</i>
<i>Cryptococcus hominis</i>	<i>Monilia krusei</i>
	<i>Monilia candida</i>

Certain strains of *Actinomyces bovis* were reported to be susceptible by Abraham and Chain<sup>2</sup> and by Herrell.<sup>67</sup> Kocholaty<sup>79</sup> stated that penicillin had a weak action against *B. abortus*. Hobby, Meyer and Chaffee<sup>73</sup> made a study of the relative susceptibility of pneumococci, streptococci and staphylococci by determining at intervals the number of survivors in a standard solution of penicillin. They found that resistance increased in the order named.

Bornstein<sup>17</sup> stated that 27 strains of enterococci representing Sherman's 4 types and 6 strains of *S. lactis* were resistant. *Mycobacterium tuberculosis* was resistant according to Abraham and Chain,<sup>2</sup> Herrell,<sup>67</sup> and Robinson.<sup>110</sup> The fact that certain organisms were susceptible and others were not stimulated Abraham and Chain<sup>1</sup> to investigate this problem. They crushed *B. coli*, and produced an extract which, upon incubation at 37° C., destroyed the growth-inhibiting properties of penicillin on *S. aureus*. This extract apparently contained an inhibiting enzyme which was called penicillinase. This substance was not present in the supernatant broth from cultures of *B. coli*<sup>72</sup> or in cultures of *S. aureus*. Apparently, penicillin was not destroyed by penicillinase but merely inhibited its action.<sup>2</sup> The presence of this enzyme may determine whether an organism is normally resistant to penicillin. Recently, Harper<sup>28</sup> found that the best source of penicillinase was the paracolon bacillus. The product prepared from this organism actively destroyed penicillin rather than merely causing inhibi-

tion. He suggested that the dried powder may be advantageously employed to inhibit the action of penicillin in blood cultures.

*Induced Resistance.* Since it was well known that organisms became resistant when exposed to sulfonamide drugs,<sup>83a</sup> the question arose whether bacteria became resistant to penicillin. McKee and Houck<sup>86</sup> passed a strain of pneumococcus (Type III) through broth containing an amount of penicillin which first permitted growth. After 26 consecutive passages, the culture became resistant to 10 times the greatest amount of penicillin which previously permitted growth; after 55 passages, 30-fold more resistant. Increase in resistance was associated with loss of virulence in mice, which was not restored by serial passages through mice.<sup>87</sup> Rammelkamp and Maxon<sup>103</sup> stated that although growing staphylococci in increasing concentration of penicillin engendered resistance, there was a reduction in the growth velocity of the resistant strains, but no penicillin-destroying enzyme was demonstrated. Schmidt and Sesler<sup>113</sup> reported that the rate and degree at which resistance to penicillin developed in pneumococci varied significantly with various strains, and that established resistance was maintained for considerable periods. The development of insensitivity *in vitro* was accompanied by a similar quality *in vivo*. Schmitzer, Camagni and Buck<sup>114</sup> found that small colony variants (G forms) of Strain 314 of *S. albus* were induced by the action of penicillin.

*Mechanism of Action.* It was agreed that penicillin was bacteriostatic,<sup>5, 19</sup> but may be bactericidal under certain conditions.<sup>28, 57, 73</sup> When sterilization of a culture did not occur, there was always a marked reduction in the number of organisms<sup>72</sup> according to Abraham,<sup>3</sup> bacteriostasis for oxygen uptake of colonies of bacteria exposed and unexposed to penicillin was the same; furthermore, staphylococci removed from the noxious effect of the drug continued to grow. Penicillin did not act like any other therapeutic agent in that it was not a detergent, not hemolytic, and was unaffected by the presence of pus, products of tissue destruction, large numbers of bacteria and para-amino benzoic acid.<sup>2, 28, 50, 57, 72</sup> In fact, the last-named substance enhanced its antibacterial effect on *B. subtilis*. Erythrocytes and plasma did not inhibit its action.<sup>102</sup> Penicillin was effective only during the multiplication of bacteria.<sup>73</sup> Pulvertaft<sup>96</sup> suggested that the effect of the drug was upon bacterial fission—not upon the metabolism and growth of the individual organism. Penicillin was not absorbed or destroyed by bacteria. With exception of staphylococci,<sup>104</sup> lysis of bacteria did not occur.<sup>73</sup> However, Gardner<sup>69</sup> noted certain morphologic changes, the more important of which were spherical enlargement of cells. Even Gram-negative organisms exhibited elongation of cells. There was no change in the structure of the meningococcus.

*Comparison of Sulfonamides With Penicillin.* Resistance to sulfonamides did not indicate resistance to penicillin.<sup>21, 84, 94</sup> McKee and Hake,<sup>89</sup> Tillet, Cambier and Harris<sup>20</sup> reported that strains of pneumococci resistant to sulfonamides were susceptible to the action of penicillin. Induced resistance of organisms to penicillin was acquired only after prolonged exposure, but resistance to sulfonamides was attained more readily. Fleming,<sup>50</sup> after studying *in vitro* the inhibitory powers of sulfathiazol and penicillin against staphylococci and streptococci, came to the conclusion that penicillin was 4 times more potent than sulfathiazol. Powell and Jamieson<sup>85</sup> conducted a similar experiment in mice infected with staphylococci, and found that the survival rate of the ones treated with penicillin was approximately twice of those treated with sulfathiazol in experimental ocular lesions caused by the staphylococcus.

The entire problem was well summarized by Florey *et al.*<sup>2</sup> who stated that while penicillin resembled sulfonamides in its bacteriostatic action, it had a number of advantages:

- (1) Its action was stronger.
- (2) It was influenced only to a minor extent by the number of bacteria to be inhibited.
- (3) It was not hydrolyzed by hydrolytic protein products or pus.
- (4) It combined low toxicity to cells with powerful bacteriostatic action.

**Pharmacology. Absorption.** Saliva did not inactivate the bitter tasting drug, but the hydrochloric acid of the stomach (not the pepsin) rapidly destroyed the active portion.<sup>98,99</sup> When the stomach was by-passed by a duodenal tube, penicillin was absorbed from the intestine.<sup>100</sup> without damage to the living cells.<sup>19</sup> Rectal administration was precluded because of the destructive action of penicillinase.<sup>1</sup> The drug was slowly absorbed after subcutaneous injection, and there was a long delay before it appeared in the blood stream. The concentration did not reach the levels attained when it was given intramuscularly or intravenously.<sup>102</sup> Intermittent intramuscular administration caused a rapid rise in the blood level, but not to the same heights as intravenous injection. The concentration tended to remain at a peak for 30 to 45 minutes. Intravenous use caused a rapid rise in the serum level, which reached a maximum immediately after injection, but then a rapid fall occurred. Traces remained for 30 to 210 minutes.<sup>102</sup> Absorption following introduction into body cavities was similar to the subcutaneous route.<sup>102</sup>

In normal subjects, penicillin was both slowly absorbed and excreted following the intrathecal injection of 5000 to 10,000 units. The absorption was more rapid in patients with meningitis.<sup>101</sup> Abraham and Chalmers<sup>12</sup> introduced penicillin intrasternally without harm, even the solid drug was introduced into a cerebral abscess without toxic effect.<sup>96</sup> Penicillin was not detected in the cerebrospinal fluid following intravenous administration.<sup>102</sup>

**Blood.** Although elevated temperatures inhibited penicillin, it was unlikely that the hyperthermia caused by febrile diseases caused any significant loss during the short time the drug remained in the body. Rammelkamp and Keefer<sup>102a</sup> reported that the degree of antibacterial action was proportional to the concentration of penicillin in the serum. Maximum effects against hemolytic streptococci were produced by concentrations of 0.019 to 0.156 Florey units per cc. of serum. Concentrations of at least 0.156 units were required for maximum bacteriostasis against *S. aureus*. A suitable level was more easily maintained by the frequent injections of smaller amounts rather than the use of larger amounts less often.<sup>28</sup>

The action of penicillin upon leukocytes is a topic of importance, for it is upon these, after bacteriostasis was produced, that the ultimate destruction of bacteria depends.<sup>2</sup> Florey<sup>48</sup> reported a fall of total leukocytes within a relative decrease in the neutrophils during the first 24 hours of the drug's administration in mice; but a regeneration was noted within 48 hours. White blood cells, as recorded by Abraham,<sup>2</sup> were killed immediately in concentrations of 1 to 100; 50% immobilized in dilutions of 1 to 250; and unharmed 1 to 500. This confirmed the earlier work of Fleming<sup>48</sup> who reported leukocytes were unaffected in penicillin solutions of 1 to 600. Leukocytes remained active in any concentration reached after intravenous administration. Penicillin did not penetrate erythrocytes,<sup>102</sup> cardiovascular and respiratory systems. Except for transient reversible depression of a cat's heart upon perfusion with penicillin solu-



tions (1 to 5000),<sup>61</sup> there was no effect on these functions within limits of therapeutic dosage.<sup>19</sup>

*Kidney.* Slight evidence of tubular damage was reported in rats.<sup>19</sup>

Occasionally, in man, there was a slightly elevated blood urea (to 35 mg. per 100 cc.) unassociated with albuminuria during the administration of penicillin. Withdrawal of the drug caused a return of the urea to normal. Turner<sup>121</sup> explained this curious fact as possibly caused by the inhibition of urease by penicillin.

*Excretion.* Some penicillin was destroyed in the body<sup>99</sup>—possibly in the liver—but the exact location remains unknown. Incubation with slices of liver, kidney, spleen, muscle and so forth caused no noticeable destruction.<sup>2,102</sup> The small amount of inactivation was related to the short time the drug stayed in the body. Rammelkamp and Keefe<sup>102</sup> estimated that 37 to 99% of the quantity administered was excreted unchanged—principally within an hour—in the urine. An excretion rate of 50% was considered to be an average figure.<sup>19,69</sup> An increased volume of urine aided in the elimination of penicillin.<sup>102</sup> Renal failure delayed excretion with a consequent rise in the concentration of penicillin in the blood.<sup>102</sup> Rammelkamp and Bradley<sup>98</sup> suggested that the rapid excretion was caused by the passage of the drug through the renal tubules as well as by the glomeruli. The administration of diodrast—known to be excreted by the tubules—decreased the loss of penicillin. The addition of such substances as hippuran or phenol red may retard the elimination of penicillin by blocking the tubules.

*Toxicity in animals.* Hobby, Meyer and Chaffee<sup>74</sup> stated that the L.D. 50 dose for an 18 gm. mouse was 32 mg. of the sodium salt. They pointed out that a cardinal symptom of toxicity was respiratory embarrassment—choking, gasping, rapid respiration. The presence of the pulmonary phenomena following toxic doses (30,000 R. U. [0.5 gm.] per kg.) into mice was confirmed by Robinson.<sup>110</sup> Hamre *et al.*<sup>62a</sup> reported that the acute toxic dose to mice, rabbits and guinea pigs was low—about 100,000 units (1 gm.) intravenously. The last-named animal was more sensitive to large doses of subcutaneous penicillin, than the other two, but within the limits of therapeutic doses (100 units per kg.), none of the animals was harmed. There was little doubt that the doses given by various investigators were large, and it was generally agreed that penicillin was relatively non-toxic in doses far exceeding those necessary for therapeutic purposes.<sup>28,88,110</sup> Although a complete histologic study of tissues of animals dying of large doses of penicillin—given rapidly or over a long period of time—has been contemplated,<sup>115</sup> none has yet appeared. However, according to one group of authors, the pathologic change most frequently noted was pulmonary congestion.<sup>74</sup>

*Toxicity.* No deaths resulted from therapeutic dosages of penicillin. Impurities remaining after extraction were believed to be the prime causes of reactions.<sup>41,54,110</sup> Since the drug now used is still considerably unfined, the elimination of foreign substances may decrease these reactions listed by Lyons:<sup>83</sup>

- (1) Chills with or without fever after intravenous injection.
- (2) Eosinophilia—20 to 30%.
- (3) Burning pain at the site of an intramuscular injection.
- (4) Headache.
- (5) Faintness and flushing of the face.
- (6) Unpleasant taste following parenteral injection.
- (7) Tingling in the testes.
- (8) Muscle cramps.
- (9) Femoral phlebotrombosis.

Certain untoward effects may be caused by the drug itself. These were grouped by the same author as follows:

1. *Urticaria*: This most common complication occurred in 5.7% of his cases. The condition, benefited by epinephrine, persisted for 3 to 5 days, then disappeared. Fever to 103° F., abdominal cramps and eosinophilia were frequent concomitants. Although it was believed that this was an allergic manifestation, sensitivity tests were negative.
2. *Fever in Afebrile Patients*.
3. *Transient Azotemia*: A rise of 5 to 10 mg. of urea per 100 cc. of blood was not uncommon. Rarely did the urea concentration exceed 35 mg.
4. *Thrombophlebitis*: This may or may not be an inherent property of penicillin *per se*. This complication has become less frequent with further purification of the drug, use of dilute solutions, and by the daily change of the needle during the use of the continuous intravenous technique.<sup>15</sup> The intrathecal injection of 10,000 units, Kammellkamp and Keefert<sup>101</sup> reported, produced headache, vomiting, increased pressure and phagocytosis in the cerebrospinal fluids; 5000 units caused only slight headache and hyperleukocytosis of the fluid.

**Administration.** Penicillin is available in vacuum-packed ampules containing 5000, 10,000, 25,000, 100,000 and 1,000,000 units.<sup>78</sup> The drug is readily soluble in distilled water, normal saline solution and 5% dextrose. The following methods of administration have been used or are now in vogue:

1. *Oral*. Florey and Florey<sup>55</sup> covered 20,000 units with cellulose acetate phthalate, and found absorption was irregular. Subsequently, this technique, together with the use of the duodenal tube, was abandoned.
2. *Continuous Intravenous*. For almost all infections—particularly the severe generalized ones—this is the method of choice. The drug is dissolved in the vehicle selected at a concentration of 50 to 100 units per cc. A needle is inserted into the vein, and anchored securely. To aid in preventing thrombosis, the needle is changed frequently. Except during the patient's sleep or delirium, the extremity need not be confined. Approximately half the daily dose is connected to the intravenous apparatus morning and evening. The first 200 cc. of the solution is given rapidly, after which then is a reduction in the rate of flow to 30 to 40 drops per minute. If there is any interruption in the flow of penicillin solutions, glucose or saline solution may be substituted, thus eliminating the necessity of removing the needle. The method is without major discomfort to the patient, and conserves the time of the medical personnel.
3. *Intermittent Intravenous*. The calculated dose is administered in a solution containing 1000 to 5000 units per cc. every 3 hours.
4. *Intramuscular*. In unskilled hands, the intramuscular injection into the gluteal or deltoid muscles is best.<sup>55, 53</sup> The solution containing 5000 units per cc. is injected every 3 hours. Some residual soreness may be eliminated by the use of saline solution instead of distilled water.
5. *Subcutaneous*. The same strength solution used for the intramuscular injection is prescribed. In both these methods, the site of injection must be changed frequently to avoid severe local reactions.<sup>62a</sup> Because and Chaffee<sup>74</sup> prepared homogeneous pellets by grinding penicillin (1 part) and cholesterol (4 parts) as well as oily suspensions made by mixing the sodium salt in water and sesame oil. While these methods have been used in animals, they are so far impractical in man. The same group of investi-

gators<sup>91,92</sup> successfully combined penicillin with certain aliphatic hydrocarbons to form esters. Their activity—both subcutaneously and orally—was undoubtedly due to hydrolysis. Although the oral dose approached the toxic dose, the margin of safety of the subcutaneous route was greater. Further work is required before this method can be fitted into the scheme of things.

6. *Topical.* Penicillin diffuses poorly, hence it is used locally in wounds, infections of pleura and joint cavities and so forth. This is in addition to the other parenteral routes listed above. The powder and highly-concentrated solutions are not used because of their highly irritating properties.<sup>21,78,110</sup> The solution of choice is isotonic saline containing 250 units per cc.<sup>78,83</sup> This may be used as a spray or by immersing gauze in it. Mucous membranes of the nose or accessory nasal sinuses are unharmed by it.<sup>16</sup> Recently, Robinson and Wallace<sup>109</sup> suggested a dressing inoculated with penicillin too crude for parenteral use. Clark *et al.*<sup>21</sup> prepared a cream composed of lanette wax, castor oil, water and penicillin especially suitable for burns.

7. *Intraspinal.* The drug has been used in solution intrathecally,<sup>13,96,101</sup> intracisternally and as the solid drug directly into cerebral abscesses.<sup>96</sup> The exact strength of the solution to be given intrathecally has not been established. However, 10,000 units (5000 units per cc. of saline) has been injected twice daily without harm.<sup>15</sup>

8. *Intranasally.* Delafeld and others<sup>99</sup> prepared a snuff composed of lycopodium (94 parts), menthol (5 parts) and penicillin (1 part). It aided in the reduction of pyogenic organisms, but was ineffectual in controlling the common cold. Sufficient clinical investigation has not been done to evaluate this type of therapy.

Bloomfield<sup>10</sup> pointed out that the administration of penicillin therapy in a hospital is best vested in a team consisting of physicians especially trained in the use of the drug plus the necessary technical help. This group evaluates the condition of the patient and prescribes the dose of penicillin. The preparation and injection of the drug together with the responsibility of keeping adequate records lies with the members of this committee.

*Therapeutics.* The expression of precise dosages is premature at the present time. With continued purification of the drug, modifications will be made. However, certain general rules have been made, chiefly by Keefer<sup>78</sup> and Lyons,<sup>83</sup> which are summarized below:

1. *Staphylococcal Infection With Bacteremia.* Although penicillin is not inhibited by toxic products, the focus of infection if surgically accessible should usually be drained. Generally, a daily dose of 200,000 to 400,000 units keeps the infection under control. As the patient improves, the dose may be gradually reduced (50,000 units a day). In assaying clinical improvement, too much regard must not be attached to the temperature chart, for lessening of pain, improvement of appetite, and a sense of well-being are equally important.<sup>55</sup> The total amount required may be 500,000 to 2,000,000 units given over a period of 7 to 14 days. Penicillin does not control staphylococcal endocarditis.<sup>83</sup>

2. *Staphylococcal Infections Without Bacteremia.* Moist dressings dipped in a solution containing 250 units of penicillin per cc. of salt solution are applied directly to the open lesions of osteomyelitis. Burns or pyogenic infections are favorably influenced by these wet dressings or a neutral cream containing penicillin.<sup>152,21</sup> The treatment of empyema requires the injection of 50,000 units in physiologic saline twice daily. In all these infections, supplemental intravenous or intramuscular injections are usu-

ally necessary. Penicillin has proved effectual locally in infections of the middle ear and of the cornea.<sup>11,12</sup>

3. *Streptococcal Infections*. Circumscribed infections are best drained, and treated with penicillin applied locally. With the exception of the resistant viridans,<sup>7,107</sup> and thermophilic (fecalis) groups, a smaller dose (100,000 units daily) inhibits this type of infection.

4. *Gonorrhea*. Mahoney and his co-workers<sup>84</sup> report that 10,000 units given intramuscularly every 3 hours for 15 doses cured this disease satisfactorily. Florey and Jennings,<sup>84</sup> Herrell<sup>88</sup> and others find that a somewhat smaller dose is satisfactory. It is generally considered that the injection of 100,000 to 150,000 units, using the continuous intravenous technique will cure almost all cases of gonorrhea.<sup>15</sup>

5. *War Wounds*. Lyons<sup>83</sup> and Thomson *et al.*<sup>119a</sup> report the largest experience to date. Because penicillin alone does not control the polymicrobial character of the wounds, surgical intervention is necessary. High concentrations of locally applied penicillin with adequate intravenous or intramuscular therapy are usually necessary. Both these excellent articles should be read in the original.

6. *Actinomycosis*. Temporary improvement has been noted and clinical trials are in the process of being carried out. Apparently not all strains are susceptible.

7. *Biliary Infections*. Since a small quantity of penicillin is excreted in the bile, the drug may be of some aid in the control of this infection.<sup>99</sup> 8. *Upper Respiratory Infection*. Antiseptic snuffs containing penicillin lessen the number of pyogenic organisms in the nose,<sup>29</sup> may have some use in the prophylaxis of diphtheria or meningitis,<sup>38</sup> but has no effect on the common cold.

9. *Syphilis*. Preliminary trials by Mahoney have shown that penicillin may be of some value in the treatment of early syphilis; but no extensive clinical reports are available. The correct evaluation is a long-range problem.

10. *Malaria*. This disease is not controlled by penicillin.<sup>83</sup>

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ent advantage that a smaller dose of it was required to produce satisfactory cholecystograms. Since that time sodium tetraiodophenolphthalein has been the cholecystographic medium of choice the world over. However, it has never been considered the ideal preparation. In spite of many improvements in the technique of administration many patients experience disagreeable reactions such as nausea, vomiting and purging; it has not been possible to administer the compound in palatable form; occasionally, at least, the reactions have been severe enough to cast doubt on the validity of the cholecystographic findings.

Researches to discover a more satisfactory compound for cholecystographic purposes have been going on from time to time since the earliest years of cholecystography. In 1927, for instance, Kirkin and Kendall<sup>10</sup> had prepared the di-iodo-di-ethyl-ether of disalicylphthalein and had administered 10 gm. of the sodium salt of this compound in 100 cc. of water to patients. Their preliminary report on the use of this substance was most encouraging but the work was discontinued, presumably because the advantages that the new compound had to offer were too few over the refined preparations of tetraiodophenolphthalein and tetrabromophenolphthalein which were being made available commercially by that time. Pribram<sup>11</sup> in 1926 had investigated a di-iodo-atropin, called biloptin, and found that while it was not very disturbing to his patients it was shown to produce renal and hepatic damage. In 1936<sup>12</sup> he reported his experience with another substance, another iodized quinoline derivative, commercially called choleselecan, but for some reason this substance was not made available for any worthwhile length of time.

No other researches along these lines were reported in the roentgenologic literature in the first 15 years after the discovery of cholecystography. Since the chemical possibilities are so numerous it is probably fair to conclude that the search for a new cholecystographic drug was not very intensive after all, and this in turn might be advanced as testimony to the generally satisfactory qualities of tetraiodophenolphthalein. Its disadvantages have proved in reality to be minor ones; experienced cholecystographic interpreters have taken them into account to make the record of cholecystography with it a truly brilliant one.

In 1940 Dohrn and Diedrich,<sup>5</sup> of the laboratories of Schering-A-G., Berlin, announced the synthesis of a new substance,  $\beta$ -(4-hydroxy-3, 5-di-iodo-phenyl)- $\alpha$ -phenyl-propionic acid, the sodium salt of which was made commercially available in Germany under the name "bileselecan." It was passed out among numerous physicians over there at once and from the very beginning reports were uniformly favorable when not enthusiastic. In the last year or two the same substance has been made available in this country by the Schering Corporation under the commercial name of priodax. Apparently many roentgenologists and other physicians have given it a trial.

Kinsel and Kinsel,<sup>6</sup> for instance, reported their experience with it in 50 unselected cases. The dose was 3 gm. stirred in a cup of tea. No obnoxious symptoms were produced in any of the cases except that one patient complained of a mild burning in the "stomach." These observers concluded that a single dose of 3 gm. of priodax administered orally produced cholecystograms equal or superior to those obtained with other cholecystographic media and that freedom from nausea, vomiting and other indications of gastro-intestinal irritation was an outstanding feature of these examinations. Of further interest in this report is the observation that a good shadow of the gall bladder was obtained in 2 cases in which

previously there had not been any cholecystographic response with sodium tetraiodophenylthalein.

Wasch<sup>18</sup> analyzed data on 134 cases in which priodax was used. In all but 33 of these a single dose of 3 gm. was administered suspended in water, fruit juice or milk. In the 33 cases a second dose of 3 gm. was given the following day, chiefly to check on the reliability of the original examination. Wasch concluded that the new compound was absorbed readily by the intestine, that its administration was simple, that unfavorable reactions were negligible, that the double dose was not necessary and that in general priodax is a more desirable cholecystographic medium than sodium tetraiodophenylthalein. He also quoted Junkmann<sup>9</sup> to the effect that because of the high alkalinity of solutions of the sodium salt of this new compound it is not practical to employ it intravenously.

Marshall<sup>13</sup> analyzed the results of the cholecystographic examination of 50 patients in which priodax was used. He gave 2 doses, each consisting of 3 gm. of the substance in tablet form, 1 after a light luncheon at noon, the other after a fat-free meal of tea and toast at 8 p.m. The tablets were swallowed whole. Marshall<sup>13</sup> too, regarded these cholecystograms as very satisfactory. He concluded that priodax had advantages over sodium tetraiodophenylthalein in that it seemed to him to be less toxic, it produced fewer disagreeable side effects, it did not act as a purgative and it seemed to be so completely absorbed in the intestine that no shadows of medium-containing intestinal loops obscured the region of the gall bladder.

These reports are encouraging. The new compound is being used quite widely now and more will be heard about it. It will be interesting to note what further experience with it will reveal. Is it actually as harmless a drug as sodium tetraiodophenylthalein has proved to be? The phenolphthalein derivatives are excreted of course mainly *via* the intestinal tract. Junkmann<sup>9</sup> stated that from 61 to 83% of the new compound is excreted in the urine within 72 hours after it is taken by mouth; this is probably too slow a rate to cause appreciable harm even to previously damaged kidneys. That it is a more palatable way of taking a cholecystographic medium can hardly be questioned. The dose (3 gm.) is less than the conventional dose of tetraiodophenylthalein (4 gm.) and a single dose seems to be adequate. Therefore, if priodax does come into more general use, it is possible that patients will no longer have to undergo the so-called double-dose technique advocated in some quarters.

If the new compound proves to be as harmless to patients as it is claimed to be, then it is possible that the cholecystographic evidence of cholecystic disease (failure of the gall bladder to accept and concentrate the media, and the manifestation of cholelithiasis) will become more reliable, if only a little. On the other hand, will the interpretation of the normal cholecystographic response have to be modified? Will more diseased gall bladders show what is now called a normal cholecystographic response with the new medium than with the old? Use of the new substance in much larger numbers of cases in which cholecystographic findings are checked carefully with pathologic and with clinical findings will supply the answers to these questions.

**Some Peculiar Forms of Pneumonia.** Pleuropulmonary Involvement in *Tularia*. Biss and Berland<sup>1</sup> studied the roentgenographic findings in the lungs and pleura in 81 cases of authentic tularemia. They mentioned the 4 recognized clinical forms of tularemia, only to note that 72 of the cases were of the ulceroglandular, glandular or oculoglandular form and 9 were classified as typhoidal.



Of the group of 72 cases there were 19 in which there was roentgenologic evidence of pathologic change in the lungs, pleura or mediastinal lymph nodes. In the earlier stages of the disease there was widening of the mediastinal shadow on the roentgenograms, indicating enlargement of the lymph nodes of the region. It seemed to these observers that when extension to the parenchyma of the lung or to the pleura took place, it did so in retrograde direction from the enlarged mediastinal nodes. Parenchymatous involvement was in the form of unilocular or multilocular consolidation, resembling typical or atypical bronchopneumonia; when the pleura became involved there was pleural effusion.

Seven of the 9 patients who had typhoidal tularemia had pneumonia and 2 of them gave evidence of hilar adenopathy. In these cases the parenchyma of the lung seemed to be involved primarily, usually without preceding enlargement of the mediastinal lymph nodes. The pulmonary lesions consisted of one or more massive consolidations, manifested roentgenographically by large homogeneous areas of density.

In the 53 cases of tularemia in which there was no evidence, clinical or roentgenologic, of pulmonary disease, there was but 1 death. Three of the 7 patients who had typhoidal tularemia and pneumonia died; 4 of the 19 patients who had non-typhoidal tularemia and pleuropulmonary complications died. It has been pointed out frequently that pulmonary complications of tularemia have grave prognostic implications; here is more evidence to show that such complications are not necessarily fatal.

Biliss and Bertrand<sup>1</sup> pointed out that symptoms referable to the thorax are often overlooked, especially when the local disease is severe and the patient is very toxemic. Two of their patients who had no respiratory symptoms and no physical signs of intrathoracic disease showed roentgenologic evidence of hilar adenopathy and patchy bronchopneumonia. It is possible that pneumonitis with or without involvement of the hilar lymph nodes is more common, even in the less severe cases of tularemia, than we think. When tularemia is complicated with pleuropulmonary disease the clinicoroentgenologic situation may be expected to be confusing, especially since the clinical evidence of respiratory involvement may not correlate well. Especially in regions where tularemia is relatively common, patients exhibiting pulmonary disease in any way atypical should receive the benefit of the diagnostic agglutination tests for tularemia.

*Pleuropulmonary Involvement in Coccioidomycosis.* Colburn<sup>2</sup> studied the roentgenologic manifestations of the pulmonary lesions of 75 soldiers hospitalized during an outbreak of coccioidomycosis which developed in association with army maneuvers in an area where the disease is endemic. The symptoms were predominantly respiratory and usually developed within 14 days after exposure. All of the patients had been exposed recently to spore-laden dust, so that the incubation period was within known upper and lower limits; all showed positive coccidioidin (intra-dermal) reactions; positive complement fixation tests or positive precipitin tests showing titers compatible with the stage and severity of disease present were shown by all, and none gave a previous history of exposure.

In all but 3 of the cases there was definite evidence of pulmonary or pleural involvement. The lesions were of several types, depending chiefly on their anatomic distribution. In 29 cases the lesion in the lung was represented roentgenographically by a fan-shaped, mottled opacity extending from the hilus, the shadow of which was larger than was considered normal; this was interpreted as interstitial bronchopneumonia with extension of the inflammatory process to the regional lymphatic

structures. In 18 of the cases the involvement seemed to be confined to the hilar or mediastinal lymph nodes. The roentgenographic evidence for this involvement was simply an increase of the hilar density. Fairly well-circumscribed, homogeneous masses in the parenchyma of the lungs, chiefly in the lower lobes, and almost always associated with abnormal hilar shadows, were observed in 20 instances. These were considered to be lobular and sublobular exudative processes, probably essentially the same type of lesion as the interstitial bronchopneumonia described previously. Cavernous lesions were observed in 3 cases and massive pleural effusion was observed in 2.

Here, too, we are dealing with an infectious agent which often produces pulmonary lesions that resemble those produced in the more familiar forms of bronchopneumonia and lobar pneumonia so closely that the roentgenologic manifestations of all of them must of necessity be of the same kind. Coccioidiomycotic pneumonitis may be considered to be a form of atypical pneumonia. Taken by themselves the roentgenologic manifestations are not distinctive, a point which Colburn<sup>3</sup> emphasized. In regions where this disease is endemic, if there is any encounter with a pulmonary lesion the clinical or roentgenologic features of which are out of the ordinary, infection with *Oidium coccidioides* must be considered among the diagnostic possibilities.

*Other Forms of Atypical Pneumonia.*—In recent years a hitherto relatively unfamiliar type of pneumonia either has become more prevalent or is being recognized much more frequently than before. Cases occur chiefly in large and small epidemics and sporadically as well. Many names, most of them indefinite etiologically, have been used in the descriptions of cases. The designation "virus pneumonia" came to be employed widely if somewhat loosely, chiefly because it was so often impossible to discover a pathogenic microorganism which could justifiably be considered to be the cause of the pneumonia that was present. Many observers, however, preferred to restrict the use of the term "virus pneumonia" to those cases in which one of the known viruses could be designated with certainty as the etiologic factor. They then used more or less descriptive pathologic terms, such as atypical pneumonia, acute pneumonitis, acute interstitial pneumonitis, focal pneumonia, acute diffuse bronchiolitis and many others like them, to identify the disease.

That a virus was a possibility, if not the probable, etiologic agent has not been denied, but the recovery of viruses is a notoriously difficult task and the facilities for the isolation and identification of them are not accessible to many physicians, so that the exact position of the viruses as etiologic agents in the cases of atypical pneumonia is being determined rather slowly. Several viruses are known to be able to produce the clinical picture, the prominent ones among them being those of influenza A and B, psittacosis, meningopneumonitis and rickettsias. As facilities for recovering and identifying these are becoming more generally available, it appears that a virus agent is being made responsible in more and more of the instances of the type of pneumonia under discussion.

Although roentgenographic findings have been included in practically all of the reports of cases, the contribution of roentgenologists to the large literature which has accumulated on the subject of atypical pneumonia has been small, conspicuously so if consideration is given to the important part the roentgenologic examination of the lungs plays in the development of the clinical syndrome. One of the prominent features of the disease is that the symptoms and physical signs of pulmonary involvement are

disproportionately small when compared with the extent of pulmonary disease shown to be present at roentgenographic examination. It is true that one of the earliest and best descriptions of the disease as it occurs in epidemic form was made by the roentgenologist Bowen<sup>2</sup> in a roentgenologic journal and an excellent description of the roentgenologic manifestations in the lungs was offered by Kornblum and Reimann<sup>11</sup> some 5 years later. Other than these there have been few, if any, formal considerations of this type of pneumonia in the American roentgenologic literature in the last decade; yet it is exaggerating only a little to say that this disease was discussed informally almost every time two or more roentgenologists got together in recent years. In 1943, however, there were several treatises on atypical pneumonia in the roentgenologic journals. There is so much uniformity in the reports that a review of all of the papers<sup>4,16,17</sup> individually seems superfluous.

McCarthy's<sup>12</sup> description of the pulmonary lesion as exhibited on roentgenograms seemed especially lucid. His experience was with 590 patients who were admitted to a station hospital of the United States Army and whose disease was classified as primary atypical pneumonia of unknown origin. Roentgenologic evidence of pulmonary disease appeared about the 4th day of illness.

According to roentgenologic findings the cases were divided into four groups. The largest number of cases fell into Group I. Early there was an increase in prominence of the hilar markings, usually greater on one side than on the other, and accentuation of the pulmonary markings. By the next day the evidence of pulmonary disease reached its maximum. In one or more lobes consolidation then developed which seemed to extend from the hilus. The consolidation was homogeneous and less dense than that of lobar pneumonia, since the pulmonary markings were visible through it. In Group II smaller, patchy areas of consolidation developed, usually in a single lobe. Some of these areas became confluent. The appearance resembled that of bronchopneumonia. In Group III the pulmonary change was in the form of thick strands radiating into the parenchyma of the lung from the hilus. When upper lobes were affected, the resemblance to pulmonary tuberculosis was so great that serial examinations were necessary to rule this condition out. In Group IV the pulmonary markings were increased throughout but in addition there were numerous or innumerable small areas of consolidation which brought the picture of military tuberculosis to mind. Patients in this group were extremely ill and the course of the disease was unusually prolonged. McCarthy<sup>12</sup> noted that pleural involvement was rare in the cases he observed.

Huford and Applebaum<sup>8</sup> reviewed the roentgenologic and clinical findings in 27 cases which they observed in civilian practice. Only the roentgenologic aspects will be given consideration here. They emphasized that while the roentgenographic evidence of pulmonary involvement was the outstanding objective feature of their cases, the findings are not distinctive enough to enable one to make an independent roentgenologic diagnosis of atypical pneumonia. Typically their patients showed diffuse, irregular, patchy densities with feathery edges extending into the pulmonary substance from the hilus. Large areas of consolidation were not common but some coalescence often took place. In several of the cases both lungs were involved.

**Comment.** From a study of the articles it is apparent that the roentgenologic manifestations of atypical pneumonia are variable and not

distinctive. In some instances the roentgenologic manifestations are of such a kind that an experienced roentgenologic observer will know at a glance that he is dealing with a disease very different from the forms of lobar pneumonia and bronchopneumonia commonly encountered; in others, perhaps in the majority, such an independent distinction cannot be made. In general it may be said that pulmonary disease becomes evident roentgenographically early in the development of the disease; that the earliest evidence is an increase of the hilar shadows, often followed by a fan-shaped, poorly circumscribed area of consolidation extending from the hilus into the substance of the lung; that the consolidation is not as dense as that encountered in lobar pneumonia and that multiple lesions are fairly common, the second lesion often developing at about the time the first was beginning to undergo resolution.

It is noteworthy that none of the observers seemed able to identify the picture of "acute tracheobronchitis" which Kornblum and Reimann described so beautifully. This should not reflect adversely on the validity of Kornblum and Reimann's findings nor on the observations that these authors made. Kornblum and Reimann had an unusual opportunity. The patients were all in one institution, all the roentgenologic examinations were made in similar very well-controlled circumstances and early medical attention was easily available. It is probable that the roentgenologic picture of "acute tracheobronchitis" is the earliest possible evidence of this pneumonia or in fact of any other type of pneumonia.

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## PHYSIOLOGY

PROCEEDINGS OF  
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA  
SESSION OF MARCH 21, 1944

**Intercorrelations Between Cardiovascular Variables in Healthy Men, and the Relation of Physique to These and Other Variables.** JAMES M. TAYNER (Hartzell Dept. of Research Therapeutics, Univ. of Penna.). In an investigation on 53 healthy students: 1. Figures for the changes in systolic and diastolic blood pressure during 20 minutes following lying down were reported. 2. The standard error of measurement of blood pressure taken by the

auscultatory technique, with certain precautions, was similar to that reported by Shock and Ogden, *z. c.*, 1.9 mm. Hg systolic and 2.84 mm. Hg diastolic.

3. Ballistocardiograms were taken 14 to 21 minutes after lying down. Aortic diameter was obtained by the Kreuzfuchs measurement. The areas under 2 complexes in each record were read. There was no significant rise or fall of mean value between the two determinations. The correlation of the two values was 0.905, with a standard error of estimation of the second from the first value of 2.48 cc., or 4.8% of the second mean value. The standard error of measurement estimated from these 2 determinations was 1.8 cc., or 3.5% of total mean value.

4. Correlations between various cardiovascular and other variables were presented. The striking thing was the lack of correlation between blood pressure and heart rate, stroke volume or cardiac output, and between cardiac output and surface area or weight. No explanation is offered at present. It is emphasized that relations between variables in a population at rest may differ entirely from relations between similar variables in a single organism under varying circumstances.

5. With the help of Dr. C. W. Dupertuis, the subjects were anthroposcopically somatographed from photographs taken as described by W. H. Sheldon. With very small numbers of limited age range, at rest, no connection was apparent between somatotype and systolic blood pressure, heart rate, ballist impacts, stroke volume, cardiac output, or cardiac output multiplied by mean blood pressure. The correlation between oral temperature and endomorphy was -0.342, and between oral temperature and ectomorphy it was +0.347 ( $P < 0.01$ ). Between respiratory rate and endomorphy it was +0.252 ( $P = 0.039$ ) and between respiratory rate and ectomorphy -0.265 ( $P = 0.032$ ). No significant correlations with mesomorphy were found.

**A Rapid Method for Estimating Serum Proteins (Formula for Calculating Serum Protein Concentration).** R. WILLIAM SUNDERMAN (William Pepper Laboratory of Clinical Medicine, Univ. of Penna.). When the differences in the refractive index of serum and water are plotted against the serum protein concentration, an excellent linear correlation is obtained. The statistically calculated regression line derived from this correlation may be expressed by the equation:

$$Pr = 510 R.I.diff. - 1.81$$

( $Pr$  = gm. of protein per 100 ml.;  $R.I.diff.$  = the refractive index of serum minus the refractive index of water.) The standard deviation for this regression line is equal to 0.31 gm. per 100 ml. The method for estimating serum protein from refractivity measurements is economical in time, easy of manipulation, requires no reagents, and is useful in guiding therapy in cases of shock.

**Topical Projection of the Cochlea to the Cerebral Cortex of the Monkey.** CLINTON N. WOOLSEY and EDWARD M. WALZ (Department of Physiology and the Otolological Research Laboratory, School of Medicine, Johns Hopkins Univ., Baltimore, Md.). The type of study previously described for

the cat (Woolsey and Walzl, *Bull. Johns Hopkins Hosp.*, 71, 315, 1942) has been extended to the monkey. Single condenser discharges were delivered through fine wire electrodes to nerve fibers at the dissected edge of the osseous spiral lamina. Amplified contralateral or ipsilateral cortical electrical responses were visualized oscillographically and photographed. The inferior and the superior banks of the Sylvian fissure were explored. In addition the auditory area was defined by click stimulation of the intact ear.

Both methods revealed that the auditory area occupies most of the inferior Sylvian bank from its caudal end to a level 4 to 6 mm. rostral to the caudal limit of the insula. Usually the area extends everywhere to within 1 mm. of the lip of the Sylvian fissure, while caudally it may spread over the lateral surface of the temporal lobe as far as the superior temporal sulcus. The area traverses the bottom of the posterior Sylvian fissure and extends for about 3 mm. onto the superior Sylvian bank posterior to the insula. The apex of the cochlea projects to the most rostral part, the middle turn to the region lateral to the posterior end of the insula and the basal coil to the remainder of the area. The cortical area for the most basal end and apparently traverses the bottom of the posterior Sylvian fissure to occupy a part of the superior bank.

In the cat a "second" auditory area, organized in a pattern inverse to that of the "primary" auditory area, was found. The presence of a similar "second" auditory system in the monkey has not yet been proved; but the evidence indicates that it exists deep within the Sylvian fissure on the lateral wall of the inferior limiting sulcus of the insula and on the dorsal bank of the posterior Sylvian fissure.

Certain homologies of the cerebral hemispheres of cat and monkey have been clarified by comparing data secured in studies of auditory, visual and somatic sensory systems of these animals.

**The Metabolism of Tryptophane by Staphylococcus Aureus as a Critical Factor in Relation to the Mode of Action of Sulfonamides.** By M. G. Sevag and M. N. Green (Department of Bacteriology, School of Medicine, Univ. of Penna.). Our studies show that the metabolism of tryptophane by resistant and susceptible strains of *S. aureus* is a keystone to understanding of the mode of action of sulfonamides. Three aspects of tryptophane metabolism have been studied: (a) tryptophane as an essential amino acid; (b) tryptophane as the precursor of one or several arylamines (p-aminobenzoic acid?) produced during the growth of staphylococci; and (c) sulfonamide-antagonist action of tryptophane.

Staphylococci must either synthesize tryptophane from other amino acids or tryptophane must be provided within the medium. Arylamine is not formed when both tryptophane and glucose are omitted from the medium. Growth takes place with or without the formation of arylamine. Largest amounts are formed under conditions least favorable for growth. Even the largest amounts of arylamine produced do not exercise anti-sulfonamide action. These facts refute the postulates by Landy *et al.* that staphylococcal resistance to sulfonamides depends upon the synthesis of larger amounts of para-aminobenzoic acid. There is as yet no chemical evidence that staphylococci synthesize or require para-aminobenzoic acid. In the presence of glucose and amino acids (tryptophane absent), with

or without pantothenic acid and riboflavin, the growth of staphylococci is inhibited by sulfonamides. The addition of tryptophane to the medium abolishes this inhibition by 50% in the absence of, and completely in the presence of pantothenate and riboflavin. These facts show that sulfonamides inhibit the oxidative-reductive reactions leading to the synthesis of tryptophane, and are incapable of inhibiting the utilization of tryptophane added to the medium. Pantothenic acid and riboflavin are participants in some manner in the metabolism of glucose and tryptophane. This is in extension of the previously formulated "inhibition of oxidative enzymes theory." (Sevag, M. G., and Shelburne, M., *J. Bacteriol.*, 43, 411, 421, 447, 1942; Sevag, M. G., Shelburne, M., and Mudd, S., *J. Gen. Physiol.*, 25, 803, 1942; Sevag, M. G., Henry, J., and Richardson, R., *Am. J. Med. Sci.*, 205, 877-878, 1943.)

# BOOK REVIEWS AND NOTICES

PSYCHOSOMATIC DIAGNOSIS. By FLANDERS DUNBAR, M.D., MEd.Sc.D., Foreword by LEONARD G. ROWNTREE, Colonel, Medical Reserve Corps, U. S. Army. Pp. 741; 15 charts, 12 tables. New York, London: Paul B. Hoeber, Inc., 1943. Price, \$7.50.

The appearance of this new volume marks another milestone in the advance of psychosomatic medicine. The concepts of this school have been widely disseminated in recent years by the journal, *Psychosomatic Medicine*, by Weiss and English's recent text-book, and by very numerous original articles. Those in touch with the medical problems of the present war, which are characterized by a high incidence of functional disorders, are finding the contribution of the psychosomatic school to be a valuable aid in the better understanding and handling of such cases.

"Psychosomatic Diagnosis" is a voluminous report on the study made by Dr. Dunbar and her associates on 1600 patients admitted to the wards of a general hospital, suffering from the following illnesses: rheumatic and cardiovascular disease, diabetes, and fracture. The admission diagnosis in each case was an organic one; Dr. Dunbar and her assistants interviewed each of these patients in order to discover the psychic component, and especially to see whether a characteristic personality pattern or socio-familial background was specific for each of the diseases studied. Such proved to be the case, and not the least surprising finding was that of a specific predisposition to accidental habit in the majority of patients on the fracture wards, which unexpectedly prevented the contemplated use of this group as normal controls. Careful personality "profiles" are presented for each group, but an indication of the author: "Hobo" for the fracture patients, "would-be top-dogs" for the hypertensives, "teachers' pets" and "martys" for those with rheumatic fever or rheumatic heart disease, and "muddlers" for the diabetics. A chapter on how to take an informative psychosomatic history adds to the practical value of this scholarly report.

THE MODERN MANAGEMENT OF COLITIS. By J. ARNOLD BARGEN, M.D., M.S., F.A.C.P., Chief of the Section on Internal Diseases, Division of Medicine, Mayo Clinic; Associate Professor of Medicine, Mayo Foundation, Rochester, Minn.; Secretary, American Gastroenterological Association; Vice-Chairman, Section on Gastroenterology and Proctology, American Medical Association. Pp. 332; 148 figs. Springfield: Charles C Thomas, 1943. Price, \$7.00.

Dr. BARGEN is indeed qualified to write a monograph on the colon because of his rich clinical and experimental experience at the Mayo Clinic. The book is timely because of the prevalence of intestinal disorders in time of war and turmoil. Anatomy and physiology of the colon, as well as methods of study of patients with colon complaints, are thoroughly discussed. There is a chapter on the "irritable colon" (mucous colitis) which presents the latest concepts concerning the etiology and treatment of this common functional disease. The organic types of colitis are divided into 9 groups: (1) Thrombo-ulcerative (streptococcal). (2) Regional (segmental) ulcerative. (3) Chronic ulcer-



five. (4) Tuberculous ulcerative. (5) Amebic ulcerative. (6) Food and vitamin deficiency type. (7) Lymphogranulomatous ulcerative. (8) Allergic. (9) Chronic ulcerative following bacillary dysentery. In each of these groups complications are thoroughly discussed.

An entire chapter is devoted to conditions to be distinguished from colitis. Diseases to be considered are: polyposis, carcinoma, regional enteritis, diverticulosis and diverticulitis, food poisoning, parasitic dysentery, non-specific granuloma, actinomycosis, appendicitis, Meckel's diverticulitis, sprue, pellagra, pernicious anemia, exophthalmic goiter, uremia, fecal impaction, foreign bodies, volvulus and intussusception, failure of rotation of the colon, benign stricture, anal infection, hemorrhoids, aggranulocytic angina, diseases of gall bladder, and urinary tract diseases.

In conclusion the author stresses that the symptoms of colitis may be a reflection of many bodily disorders extrinsic to the bowel.

L. LAT.

**PATHOLOGY AND THERAPY OF RHEUMATIC FEVER.** By LEOPOLD LICHTWITZ, M.D., Latey, Chief of the Medical Division of the Montefiore Hospital, and Clinical Professor of Medicine, Columbia University, New York City. Foreword by WILLIAM J. MAJOR, M.D., L.L.D., F.R.S. (Edin.), Consulting Neurologist to the City Hospital; Formerly Professor of Nervous and Mental Disease, Fordham University, New York City. Edited by MAJOR WILLIAM CHESTER, M.C. Pp. 211; 60 figs. New York: Grune & Stratton, Inc., 1944. Price, \$4.75.

This is a very dangerous book. It is written in the authoritarian manner, characteristic of continental writers, which unfortunately is often accepted at its assumed value by the careless reader. In this instance this acceptance might the more readily happen, since in the foreword the author is named "our foremost authority on the pathology of function."

An author, of course, has a right to state his opinions but only as opinions, not as facts. In this instance the author accepts the view that rheumatic fever is caused by sensitization to antigens, but he widens the list of offending antigens to include both non-pathogenic organisms and foreign proteins "not of microbic origin." The rôle of the streptococcus is not stressed—in fact it is scarcely touched on; nor does the word "streptococcus" appear in the index. This leads to some absurdities such as the inclusion of epidemic pleurodynia as a rheumatic manifestation in the nervous system, and attributing this disease to previous vaccination or some other active immunization. Also, the flat acceptance of overgrowth of fingers and big toes as a result of "chronic" rheumatic fever is a good example of the uncritical fashion in which material is included.

It is the hope of the Reviewer that these comments will lead the reader of the book to use his critical judgment throughout.

P. P.

**MEDICAL PARASITOLOGY AND ZOOLOGY.** By RALPH WEIRY NAVES, B.Sc., M.D., Dr. P. H., Assistant Professor of Public Health and Preventive Medicine, Cornell University Medical College; Consulting Parasitologist, New York Hospital, Fellow, American Public Health Association; Lieutenant-Colonel and Flight Surgeon, Medical Reserve Corps, U. S. Army. Foreword by JOHN C. TORREY, Ph.D., Professor (Emeritus) of Epidemiology, Cornell University Medical College. Pp. 534; 95 figs. New York, London: Paul B. Hoeber, Inc., 1944. Price, \$6.00.

This book is said to be an attempt to present essentials in brief form and so to replace larger and more complete texts now available. Less extensive it is, but claims to conciseness cannot be allowed. For example, what is a non-migrating *Endamoeba coli* (p. 13) and why is this "a comparatively innocu-

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ous species" (p. 17)? It is non-pathogenic without equivocation. The statement "The cyst is usually the only form in which *B. histolytica* remains viable after passing the normal acid stomach" implies rather gross fecal contamination of food, and by comparatively fresh feces at that (p. 26). Interpretations of the development of amebiasis in man (pp. 28 and 29) draw unwarranted assumptions from experimental infections in cats, and in this section the statement of incidence in spite of abundant records. Further, there is no indication of the significance of *Trichomonas vaginalis* infection, and the admonition that transifers of this organism to mouth and rectum be prevented is a bit excessive in view of recent experiments. On page 59, organisms of the genus *Leishmania* are said to be transmitted through feces of insects when those which occur in man are certainly not. "Endothelium" is substituted for macrophage cells (p. 62), "leukemia" is given as a symptom of kala-azar instead of leukopenia (p. 65), and animal inoculation is suggested as an aid to diagnosis of this disease when it is well known that few animals are susceptible and incubation is prolonged. Occurrence of *Leishmania donovani* in oral and nasal secretions is not mentioned, yet this seems to be an important factor in spread of infection. On page 90 "merocyte" is used for merozoite. In several parts of the section on malaria sporozoites are said to invade erythrocytes when all evidence is against this. The exoerythrocytic phase of Plasmodium becomes "extra erythrocytic" (p. 113). Also to be questioned is the statement that *Ascaris lumbricoides* "sometimes (becomes) attached to the mucosa, which they are probably able to pinch off in small masses" (p. 147), and there is no clear statement of how hookworms cause anemia.

These are not isolated instances; many others could be cited. They illustrate rather marked incoherence, carelessness and poor organization. Everything contained in the book is presented in better form elsewhere, and the glossary is no substitute for a dictionary.

H. R.

PIONEERS OF PEDIATRICS. By ABRAHAM LEVINSON, M.D., B.S., Assistant Professor of Pediatrics, Northwestern University Medical School; Professor of Pediatrics, Cook County Graduate School of Medicine; Attending Pediatrician, Children's Division of the Cook County Hospital; Senior Attending Pediatrician, Sarah Morris Hospital for Children of the Michael Reese Hospital; Senior Attending Pediatrician, Mount Sinai Hospital, Chicago. Second ed., revised and reset. Foreword by Isaac A. Abr. 15th Publication in *Historia Medicinæ* series. Pp. 119; many pictures. New York: Froben Press, 1943. Price, \$2.00.

This second edition, appearing 7 years after the first, is the 18th Publication in the *Historia Medicinæ* series of the Froben Press. Professedly "a bird's eye view of pediatric progress as reflected in the contributions of pioneers in the field," this compendium should be evaluated on the basis of wisdom of selection, accuracy and readability, rather than for completeness and reference value. One comes regretfully to the conclusion that at least two of the three qualities sought for have not been attached. Perhaps the attempt to cover too much ground has been the cause of a dry condensed style that, for the most part, is even less entertaining than the average catalogue raisonne. As to selection, while personal comparisons, which are especially apt to be odious and fruitless, will not be made here, the Reviewer must express a general opinion that there are many errors of both omission and commission in the "pioneers" selected. However, while most readers will prefer to go to Rubrah's excellent source book, "Pediatrics of the Past," for their historical personages in pediatrics, this more recent production also has its field. Finally, the list of American contributions and the 4½ page bibliography are useful but do not compensate for the lack of an index.

E. K.

**THE MARCH OF MEDICINE.** New York Academy of Medicine Lectures to the Laity, 1943. Pp. 151; no illus. New York: Columbia University Press, 1943. Price, \$2.00.

This 8th Series of the Academy's Lectures to the Laity demonstrates how widely the scope of the series has been enlarged since their beginning. No longer merely descriptive of this or that disease or group of diseases, they treat, this year, for instance, of such topics as Crime and Punishment (Bernard Glueck); The Scientific Method and Our Plans for Peace (Sir Norman Angell); War and Medicine (Edgar Erskine Hume); Aggressiveness—Individual and Collective (Franz Alexander); Let Babies Be Our Teachers (Myrtle McGraw); and Nature and Man, The Linsly R. Williams Memorial Lecture (Robert R. Williams). Thus, psychiatric problems—human behavior—and the relation of medicine and science to society and the world crisis appropriately dominate the series, though not to the exclusion of more directly scientific subjects such as enzymes and historical medicine, for instance. To those already acquainted, the series needs no endorsement; those yet unacquainted can be assured that an evening's reading will not go unrewarded.

E. K.

**ESSENTIALS OF DERMATOLOGY.** By NORMAN TOBIAS, M.D., Senior Instructor in Dermatology, St. Louis University; Assistant Dermatologist, Firmin Desloge and St. Mary's Hospitals; Visiting Dermatologist, St. Louis City Sanatorium and Isolation Hospital; Fellow, American Academy of Dermatology and Syphilology; Diplomate, American Board of Dermatology and Syphilology. Second ed. Pp. 497; 143 figs. Philadelphia, London, Montreal: J. B. Lippincott Company. Price, \$4.75.

The present Reviewer had the pleasure of reviewing the first edition of this now popular work (Am. J. Med. Sci., 202, 276, 1941) and recommending it "for the student and general practitioner who does not have the facilities nor the inclination to consult a larger volume." In the second edition, most of the criticisms leveled against the first edition have been scrupulously heeded. Many portions have been rewritten or considerably elaborated for the sake of clarity. Close attention has been paid to certain live subjects, such as vitamin and sulfonamide therapy and the new ointment bases. Nineteen additional photographs have been included in the revision. A new feature of this edition, not mentioned in the Preface, is the utilization of the inside of the covers and the fly-leaves for a compilation on the sulfonamide drugs in dermatology and a listing of the normal values of various blood constituents and of various tests. The Reviewer again recommends this compend as one of the best of the shorter works on dermatology.

H. B.

**HEALTH AND HYGIENE.** A Comprehensive Study of Disease Prevention and Health Promotion. By LLOYD ACKERMAN, Western Reserve University. Pp. 895; 59 figs; 27 tables. Lancaster, Pa.: The Jacques Cattell Press, 1943. Price, \$5.00.

This book attempts to cover the main problems of hygiene in terms which should be understandable by the general public. The author has succeeded remarkably well in meeting this difficult assignment and has produced a book of considerable merit. He believes that comprehensive courses in hygiene should be given to college students in their junior and senior years, and the book should have great value for such a purpose. It also should prove very useful to educated members of the general public and enable them to understand the elements of prevention of infection, vaccination, and the development of immunity, as well as nutrition and social health. Considerable attention is given to the evolution of health concepts with an account of the development of medicine from the early days of *Asclepius* through *Paracelsus* to the present day. The position of special cults such as *allopathy*, *homeopathy*,

osteopathy, chiropractic and Christian Science in this development is indicated. There are interesting commentaries on the common factors in homeopathic, Christian Science and *Abscultural* practices. The author quotes original sources, and references may be found in this book to "Christian" religious literature from Genesis to Mrs. Eddy. With this evidence the reader should be able to form opinions on the virtues and weaknesses of various cults.

Throughout the book the author has attempted to present the main pertinent facts, while allowing the reader to draw his own conclusions. Sometimes he might be accused of giving a biased presentation of the data, but for the most part, the facts are simply stated so that logical deduction seems obvious. The sections on mental health, reproduction and drug action (including the effects of alcohol and nicotine) are written in a frank and interesting manner. On such controversial matters, however, the author is bound to find disagreement among his readers, though this Reviewer would quarrel with little of it. No sane reader should be offended by the unbiased presentation of the basic facts. For student use, the inclusion of a large section on psychology seems questionable to this Reviewer. It is not always advisable to encourage introspection in either early or late adolescence, though if introspection has already started, these sections should prove useful. The various chapters include lists of literature which should enable a student to sample original sources and to follow the present trends of development. Useful tables are given of the nutritional values and compositions of various foodstuffs, as well as data on their vitamin content. While the book covers a field held from vital statistics through nutrition to psychology, the views presented appear to be up-to-date and to be well supported by references to recent literature.

H. B.

**BIOLOGICAL SYMPOSIA.** Edited by JACQUES CATTELL, Editor of "The American Naturalist" and "American Men of Science." Volume X. Frontiers in Cytochemistry. The Physical and Chemical Organization of the Cytoplasm. Edited by NORMAN L. HOERN, Professor of Anatomy, School of Medicine, Western Reserve University. Pp. 334; 105 figs. Lancaster, Pa.: The Jacques Cattell Press, 1943. Price, \$3.50.

This tenth volume of Biological Symposia is presented as a tribute to Professor R. R. Bensley, Professor Emeritus of Anatomy of the University of Chicago, and includes 15 papers covering much of our present knowledge of cellular structure. The contributions include discussion of the chemistry of cytoplasm (electrolytes, nucleic acids, nucleoproteins, minerals), the morphology of cytoplasm (cytoplasmic components, ultrastructure, macromolecular particles), the respiration of cells (biogenic oxidation-reduction reaction systems), and the pathology of certain types of cells (cancer cells and degenerating motoneurons). The material in this book represents the groundwork upon which important new medical discoveries may well be based. It should be a valuable addition to the libraries of those who are actively seeking further understanding of cellular functions. The printing and illustration of this volume follows the high standard set in previous editions of Biological Symposia.

posita.

**PRIMER CONGRESO NACIONAL SOBRE ENFERMEDADES ENDEMO-EPIDEMICAS.** First National Congress on Endemo-epidemic Diseases, held at the "Instituto Jose Penna," School of Medical Sciences of Buenos Aires, November, 1942. Pp. 679; many tables and figs. Buenos Aires: Revista Oral de Ciencias Medicas, 1943.

The articles reported in this volume were presented at the First National Congress on Endemo-epidemic Diseases held at the "Instituto Jose Penna" under the auspices of the School of Medical Sciences of Buenos Aires. The Congress consisted of eight sessions, devoted to problems of diphtheria;

exanthematic typhus and other diseases recently found in this part of the continent; brucellosis and poliomyelitis; and diseases of the respiratory tract. The last three sessions were allocated to all other diseases. The sessions on diphtheria included articles on the different clinical manifestations, prophylaxis, treatment, epidemiology, as well as bacteriologic studies which comprised immunity, pathogenicity, allergy, relationship between clinical form of the disease to the bacterial type of the diphtheria bacillus, and so forth. In the meeting on exanthematic typhus papers were also read on yaws, "fevere bouton-neuse," and "mal de pinto," at the meeting on brucellosis papers on hidatidosis and Chagas' diseases, a social problem in South America, were also included. The diseases of the respiratory tract considered were the pneumonias and influenza. Problems of prophylaxis, immunity, diagnosis, treatment and epidemiology were discussed. In the final sessions papers on bacillary dysentery, bubonic plague and trichinosis were read.

The book is a valuable aid in comprehending the work that is being done in Argentina and the progress toward the solution of the problems involved. Most of the articles do not contain a bibliography, an omission that limits the usefulness of the book for those seeking further information. Some scientists from Uruguay collaborated in the Congress, and, consequently, their papers are included in the volume.

L. G.

**QUANTITATIVE PHARMACEUTICAL CHEMISTRY.** By GLENN L. JENKINS, PH.D., Professor of Pharmaceutical Chemistry, College of Pharmacy, University of Minnesota, and ANDREW G. DU MEZ, PH.D., Professor of Pharmacy and Dean of the School of Pharmacy, University of Maryland. Second ed., 6th impression. Pp. 466; 67 figs. New York and London: McGraw-Hill Book Company, Inc., 1937.

This is a well-written book for students of pharmacy, and outlines clearly the methods used in official pharmaceutical analyses. It is to be regretted, however, that this pharmacy text does not concern itself more with the newer pharmacologic agents and less with the older, infrequently used drugs. A new edition including the changes incorporated in the *U. S. Pharmacopoeia XII* and *National Formulary VII* could correct this failing.

J. C.

**ORAL PATHOLOGY.** A Histological, Roentgenological, and Clinical Study of the Diseases of the Teeth, Jaws, and Mouth. By KURT H. THOMA, D.M.D., Professor of Oral Surgery and Bracket Professor of Oral Pathology, Harvard University; Oral Surgeon and Chief of Dental Service, Massachusetts General Hospital; Oral Surgeon to Brooks Hospital; Dental Surgeon to Dental Department and Consultant in Oral Surgery to Tumor Department, Boston Dispensary and Joseph H. Pratt Diagnostic Clinic; Consulting Oral Surgeon, New England Baptist Hospital; Consulting Oral Surgeon, Beth Israel Hospital. Second ed. Pp. 1328; 1388 figs. (128 in color); 49 tables. St. Louis: C. V. Mosby Co., 1944. Price, \$15.00.

We are glad to see the second edition of this valuable work, but suggest a more precise title for future editions. To quote from the Preface to this edition, the sentiment of which we endorse—"The surprisingly great demand which rapidly depleted the first edition of *Oral Pathology* has made it necessary to bring out this second edition. The interest which the profession has taken in this book, the content of which is almost purely scientific, is an auspicious indication of the trend in dentistry. Not only the student, but also the man who has been in practice for years, feels the need for the scientific background which the study of pathology alone can give. Thus dentistry has recognized the value of basic knowledge as a foundation for its clinical development. The ability to diagnose and treat disease intelligently is based on a thorough understanding of both disease processes and their

many and varied causes. . . . additions have been made to include a few of the rarer diseases that are omitted from the first edition and to bring the book up to date. A number of new illustrations have been added and others graphically (such as on p. 848, iii; p. 1270, l, 14).

E. K.

**BIOMICROSCOPY OF THE EYE.** SLIT LAMP MICROSCOPY OF THE LIVING EYE. Vol. I. By M. L. BERLINER, M.D., Assistant Professor of Clinical Surgery (Ophthalmology), Cornell University Medical College; Associate Attending Surgeon, New York Hospital; Assistant Surgeon, New York Eye and Ear Infirmary; Instructor in Biomicroscopy, Post-Graduate School, New York Eye and Ear Infirmary; Senior Associate Attending Ophthalmologist, Beth Israel Hospital, New York. Pp. 709; 512 illus. (40 pages color plates). New York, London: Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, 1943. Price, \$17.50.

This is the first appearance in America of an original volume on slit-lamp microscopy of the human eye. The chapters include a history of the development of biomicroscopy, the technique of biomicroscopy and the normal findings in the anterior segments of the globe exclusive of the iris, vitreous and lens. Developmental anomalies, inflammatory lesions, traumatic injuries and degenerations, and dystrophies are then considered in the various chapters. The text is excellent and the illustrations are by far the best that have appeared from an American press. The book is undoubtedly on a par with that of Vogt, and it will be warmly welcomed by all American ophthalmologists.

F. A.

**BACKACHE AND SCIATIC NEURITIS.** By PHILIP LEWIN, M.D., F.A.C.S., Associate Professor of Bone and Joint Surgery, Northwestern Univ. Medical School; Attending Orthopedic Surgeon, Cook County Hospital; Attending Orthopedic Surgeon, Michael Reese Hospital; Professor, Orthopedic Surgery, Cook County Graduate School of Medicine, Chicago; Lieutenant Colonel, Medical Corps, U. S. Army. Pp. 745; 235 figs. Philadelphia: Lea & Febiger, 1943. Price, \$10.00.

This is a very difficult book to review, for Dr. Lewin has attempted to compress an amazing amount of information in his book. His charts and diagrams at the beginning of many of the chapters show originality and disclose a method that could be well utilized in the teaching of students. These are very ingenious and informative. In the cuts the author is remarkably clear; they deserve high praise. The Roentgen rays, photographs and line drawings and anatomic pictures are consistently good and show a tremendous amount of work and meticulous care in assembling. The book can be recommended as being up to the minute in the great diversity of conditions that are considered in the very difficult subject of backache and sciatic neuritis.

P. C.

**ON THE INFLUENCE OF TRADES, PROFESSIONS, AND OCCUPATIONS IN THE UNITED STATES, IN THE PRODUCTION OF DISEASE.** (Reprinted from *Transactions of the Medical Society of the State of New York*, 3, 91, 1837.) By BENJAMIN W. MCCREADY, M.D. Introductory Essay by GEORGE W. MILLER, M.A. (Publications of the Institute of the History of Medicine, The Johns Hopkins University. Fourth series: Bibliotheca Medica Americana, vol. 4). Pp. 127. Baltimore: The Johns Hopkins Press. Price, \$1.75. After a series silence of several years, the Johns Hopkins Institute of the History of Medicine now publishes a fourth volume of the Bibliotheca Medica Americana. The McCreedy text of a century ago is much less important than Thacher's,

John Morgan's and Wm. H. Welch's that constitute the first 3 of the series (see *Am. J. Med. Sci. Review*, 195, 370, 1938). It is also much less well known; so that, if it is as meritorious as Miss Miller's Introduction indicates, a reprint is a useful addition to American medical historical sources.

Written as a prize competition essay when the author, aged 23, was but 2 years out of medical school, the successful essay was published in an obscure journal with a circulation of but 100 copies. It is unlikely, in Miss Miller's phrase, that it "ever attracted any attention or had any influence." This first treatise on occupational diseases written in this country, then, is chiefly valuable as a good picture of the subject 100 years ago for the use of future medical historians. The author went on to the life of a busy practitioner and medical teacher in New York City. He died in 1892 without contributing further to the subject of his essay.

**A SYNOPSIS OF SURGICAL ANATOMY.** By ALEXANDER LEE MCGREGOR, M.Ch. (Edin.), F.R.C.S. (Eng.), Lecturer on Surgical Anatomy, Univ. of Watersand; Assistant Surgeon, Johannesburg General Hospital. Fifth ed. Foreword by Sir HAROLD J. STILES, K.B.E., F.R.C.S. (Edin.). Pp. 710; 696 illus. Baltimore: Williams & Wilkins, 1943. Price, \$6.50.

The fact that this small volume is now in its 5th edition is mute testimony to its wide popularity. The emphasis on the practical and functional aspects of anatomy makes it especially useful as a quick reference in clinic or operating theater. The first half deals with the anatomy of the normal, the last half with the abnormal. (The semi-diagrammatic line drawings with which the volume is profusely illustrated serve to aid in fulfilling these objectives.) In the latter section of the book the Author brings out the signs through which evidence of anatomic abnormality may be elicited.

The value of the work is further enhanced by the inclusion of many bibliographic references to special articles—and this new edition serves to bring these references up to date. Outline form, with economy of words, is employed throughout. As a supplement to standard works on anatomy, this book will be found very useful by students and practitioners of surgery.

J. L.

**CLINICAL TROPICAL MEDICINE.** By Twenty-seven Authors. Edited by Z. TAYLOR BECOVITZ, M.D., Ph.D., F.A.C.P., Assistant Clinical Professor, New York Post-Graduate Medical School, Columbia Univ.; Physician in Charge, Parasitology Service, Department of Health, City of New York; Consultant in Tropical Medicine, Ellis Island Hospital, U. S. Public Health Service. Foreword by WILLIAM A. SAWYER, M.D., Director, International Health Division, Rockefeller Foundation. Pp. 957; 121 figs; 11 tables. New York, London: Paul B. Hoeber, 1944. Price, \$14.00.

In keeping with the present trend toward edited works, this most recent American book on tropical diseases has 27 contributors, all well qualified. The bulk of the material, however, was prepared by the Editor, Z. T. Becovitz, C. F. Craig, Henry Pinkerton, Howard Fox, E. Vedder and Morris Moore. Except for the first section, which deals with the diarrheal diseases and includes amebic as well as bacillary dysentery and cholera, the subject matter is arranged according to etiologic agents. This in itself will be a great aid to the novice. The sections include: diseases caused by blood protozoa, by spirchetes and spirochaetes, by rickettsia; viral diseases, bacterial infections and nutritional disturbances. Also included are sections dealing with diseases caused by yeasts and fungi, infestation by helminths, tropical snakes and poisonous insects, and a concluding section on the effects of heat, hygiene and sanitation. The individual diseases are considered under the following headings: historical note, etiology, epidemiology, pathology, symptomatology, complications, laboratory and differential diagnosis, treatment and prophylaxis. Of

particular interest is the detailed discussion of laboratory procedures which often, *e. g.*, in amebic dysentery and protozoan diseases of the blood, can be carried out by the physician himself. An excellent series of 10 plates on the intestinal protozoa, as well as plates devoted to the malarial parasites and eggs of the helminths will prove useful. The clinical photographs are on the whole quite good. Though several microphotographs of tissue lesions are included, their number might well be greater. This because most men who will use the book never had formal instruction in the pathology of these diseases and hence will find it difficult to form a mental picture from the written description. The discussions on therapy are brief, yet adequate. One is struck by the number of specific drugs which are available for treating these diseases. However, realizing that they are parasitic rather than degenerative in character this should not occasion undue surprise in our era of chemotherapy. The volume is well bound and printed; the paper is of good quality. This book should prove of value not only to physicians in the armed forces, but to the general practitioner as well.

H. S.

THE CHEMISTRY OF ORGANIC MEDICINAL PRODUCTS. By GLEN L. JENKINS, Dean and Professor of Pharmaceutical Chemistry, School of Pharmacy, Purdue University; and WALTER H. HARTUNG, Professor of Pharmaceutical Chemistry, School of Pharmacy, The Univ. of Maryland. Second ed. Pp. 675; 71 tables; many small figs. New York: John Wiley & Sons, 1943. Price, \$6.50.

The organic compounds of the *materia medica* have been classified into groups according to chemical function, some 14 classifications being discussed. Thus their chemistry, their preparation and properties can be correlated effectively. The therapeutic uses are indicated in many instances, and some idea is given of the relative importance of the various compounds. The text is an excellent presentation of the rôle of organic chemistry in the formulation and development of medicinal products. It should be of particular value to students and workers in fields related to medical science. This 2d edition has been greatly improved by a complete revision. Some chapters have been rewritten, and a new chapter discussing "Some Physico-chemical Properties of Medicinal Products" has been included. It is quite up to date.

H. V.

THE PERMEABILITY OF NATURAL MEMBRANES. By HUGH DAVSON, D.Sc., Demonstrator in Biophysics and Beit Memorial Fellow, University Coll., London, and JAMES FREDERIC DANIELLY, D.Sc., A.I.C., Beit Memorial Research Fellow and Fellow of St. John's Coll., Cambridge, England. Foreword by E. NEWTON HARVEY, Professor of Physiology in Princeton Univ. Pp. 361; 73 figs.; 72 tables. Cambridge: University Press; New York: Macmillan, 1943. Price, \$4.75.

This timely book brings together the significant material that has resulted from studies upon permeability of many writers during the last 20 years. The book is comprised of 21 chapters and an Appendix. The first chapter deals with the significance of permeability studies and the second with methods of studying membrane permeability including chemical, electrical, and radio-active isotope methods. Chapter 3 presents a discussion of equilibrium conditions of cells, and Chapter 4, the more important equations used in permeability studies. In later chapters the process of diffusion, the structure of the plasma membrane, and the interpretation of permeability measurements are considered. Permeability to non-electrolytes, gases, water, proteins and lipoids, ions and weak electrolytes and dyes are covered in the succeeding chapters. Impedance, potential measurements, the effect of narcotic substances and temperature are followed by chapters on hemolysis and the membrane permeability to secretion. A chapter is devoted to the function of



on the theory of penetration of a thin membrane completes the book. The advance in our knowledge of permeability has depended largely upon the contributions of surface chemistry and the physico-chemical structure of liquids and solids. In this book, written by two men who have contributed a great deal to our knowledge of the subject, the relevant and significant material has been carefully selected from the mass of data upon the subject. In addition, a valuable bibliography follows each chapter. Everyone interested in biologic processes should certainly have this book at hand. When it is coupled with Bourne's recent "Cytology and Cell Physiology," a comprehensive survey of our modern concepts of the cell and its functions is obtainable.

AN OUTLINE OF GENERAL PHYSIOLOGY. By L. V. HEILBRUNN, Ph.D., Professor of Zoology, Univ. of Penna. Second ed., revised. Pp. 748; 135 illus. Philadelphia and London: W. B. Saunders, 1943. Price, \$6.00.

For several years Heilbrunn's "General Physiology" has enjoyed a wide circulation as a text for college students and a work of reference for others interested in the subject. The 2nd edition, enlarged and revised to the extent of being virtually a new book, will be of increased usefulness to both classes of readers. The ground covered by it is General Physiology in the broad sense of Bayliss in his "Principles of General Physiology" rather than in the more restricted one of the *Journal of General Physiology*. Included with more conventional material are such up-to-the-minute topics as the electron microscope and the use of tracer elements. Not the least valuable feature of the book is a well-selected list of over 4000 references to the literature accompanying the text in convenient footnote form. The college student, the general reader interested in physiology, and the specialist will all find in it much that is useful and stimulating.

MEDICAL PHYSICS. Editor-in-Chief, OTTO GLASSER, Ph.D., Head, Department of Biophysics, Cleveland Clinic Foundation; Professor of Biophysics, Frank E. Bunts Educational Institute; Consulting Biophysicist, University Hospitals of Cleveland, Cleveland, Ohio. Pp. 174; many figs. and tables. Chicago: Year Book Publishers, 1944. Price, \$18.00.

Is one who first looks over this huge volume to marvel more at the boldness of the publisher who conceived it—a pioneer of its kind—and then published a heavy \$18 book in one volume, or at the energy of the Editor who with the aid of 23 Associate Editors and over 200 Collaborators selected and provided the text? It is not surprising that manuscripts were being turned in to the Editor from early 1941 to October, 1943. The Collaborator group includes a number of well-known authorities in their fields, which cover a wide range: Physics, Chemistry, Biology, Physiology, Pathology, Internal Medicine, Surgery, Neurology, Public Health and others too numerous to mention. The 200 or more items found in the Table of Contents are arranged alphabetically as in an encyclopedia. In the Classified Table of Contents, including 24 topics, from Anatomy to Urology, of course the subdivisions do not follow the paginated sequence.

The book "attempts to embody a combination of an *encyclopedia*, sufficiently comprehensive to serve as a reference for all those whose occupations involve any aspects of medical physics; a *textbook*, adequately detailed in exposition to serve students; and a *working instrument*, in which may be found the data necessary for actual application of the principles of physics to medicine. Readers will undoubtedly discover that this volume falls short of the attainment of that ideal." We regret to have to say that the last sentence is only too true, for various reasons that need not be gone into in detail. However, there is more than enough of value in the text as it stands to make it

well worth while, and the same vigorous editing can doubtless correct errors of omission and commission in future editions. And by "errors of commission" it is not intended to mean inaccurate statements; there are many sections that seem unnecessary, even out of place in a work of this kind. As one might expect, some of the articles are inferior (poorly selected, poorly planned, and poorly carried out); but many more, especially if one takes the standing of the author into account, are just the opposite. Medical men coming to the book for information and instruction of a medico-physical nature will as a rule be well satisfied. One has only to compare the first edition of the *Encyclopædia Britannica* with the eleventh or fourteenth to visualize a far-reaching future for this first venture of its kind in Medical Physics.

E. K.

BEHIND THE UNIVERSE: A Doctor's Religion. By Louis BERMAN, M.D. Pp. 308. New York: Harper & Brothers, 1943. Price, \$2.75.

The author, an endocrinologist, states that man, the only species cognizant of his place and functions in the universe, will become extinct unless a way is found to end this ever-increasing destruction by his fellows. The remedy offered in this thesis is disclosed in the following chapters on the immortality of ideas, the isolation of individual consciousness, the continuity of the life personality, the brain as the medium of continuity, psychic forces in life history, the creative powers of the unconscious, from the unconscious to the superconscious, a world prepared for life, the universe as a psycho-continuum, the cosmic drive behind consciousness, the living as a multiple split personality, the return to the mystic participation and the God of evolution.

From protoplasm to man, and finally to God, many topics are discussed—a range too great for comprehensive review. We are told how symbiosis—mutualism—is at work between plant and animal, between plant and animal, also between animal and animal. The life of the bee is cited, where though each insect has its own job, all work to maintain "the spirit of the hive." In the case of man, it is claimed that his "chronic unhappiness has been due to the poisonous privacies and destructive discontentments bred of individuality." A universal psychic symbiosis, a mystic participation, and a renaissance of God-consciousness is offered for the salvation of the race. The author's style includes many well-chosen metaphors. Though some may question the soundness of his doctrine, his thesis affords much interesting and stimulating reading.

N. Y.

## NEW BOOKS

*Clinical Lectures on the Gallbladder and Bile Ducts.* By SAMUEL WEISS, M.D., F.A.C.P., Clinical Professor of Gastroenterology, New York Polytechnic Medical School and Hospital; Gastroenterologist, Jewish Memorial Hospital, New York; Consulting Gastroenterologist, Beth David Hospital, New York, Long Island, etc. Pp. 504; 124 figs.; 21 tables. Chicago: Year Book Publishers, 1944. Price, \$5.50.

*Der Schwund Tuberkulöser Lungenkavernen.* By PROF. DR. WALTER BERLINGER. Pp. 130; 61 illus. Basel: Benno Schwabe, 1943. Price, 15 Swiss Fr.

*The Jews and Medicine. Essays.* By HARRY FRIEDENWALD, M.D., D.H.L. (Hon.), D.Sc. (Hon.), Professor Emeritus of Ophthalmology, Univ. of Maryland. Vols. I and II. Pp. 817; a few figs. Publications of the Institute of the History of Medicine, Johns Hopkins Univ. First series: Monographs, Vols. II and III. Baltimore: Johns Hopkins Press, 1944. Price, \$7.50.

*The Riddle of Cancer.* By CHARLES OBERLING, M.D. Translated by WILLIAM H. WOLOGOW, M.D. Pp. 196. New Haven: Yale Univ. Press; London: Oxford Univ. Press, 1944. Price, \$3.00.

- Vascular Responses in the Extremities of Man in Health and Disease.* By DAVID I. ABRAMSON, M.D., F.A.C.P. Pp. 412; 59 figs.; 4 tables. Chicago: Univ. of Chicago Press, 1944. Price, \$5.00.
- Handbook of Nutrition.* A Symposium Prepared Under the Auspices of the Council on Foods and Nutrition of the American Medical Association. Pp. 586; many figures and tables. Chicago: American Medical Association, 1943. Price, \$2.50.
- History of Miners' Diseases.* By GEORGE ROSEN, M.D. Introduction by HENRY E. SIEGIST, M.D. Pp. 490; many figures and tables. New York: Schuman's, 1943. Price, \$8.50.
- A Bio-Bibliography of Andreas Vesalius.* By HARVEY CUSHING, M.D. Preface by JOHN F. FULTON, M.D. Publication No. 6 Historical Library, Yale Medical Library. Pp. 229; 89 figs. New York: Schuman's, 1943. Price, \$15.00.
- Harvey Cushing Collection of Books and Manuscripts.* Publication No. 1 Historical Library, Yale Medical Library. Preface by JOHN F. FULTON, M.D. Pp. 207. New York: Schuman's, 1943. Price, \$8.50.
- An Atlas of Anatomy.* By J. C. BOURLEAU GRANT, M.C., M.B., F.R.C.S. (Edin.), Professor of Anatomy in the Univ. of Toronto. Vol. II. Vertebral and Vertebral Column, Thorax, Head and Neck. Pp. 390; 460 figs. Baltimore: Williams & Wilkins, 1943. Price, \$5.00.
- The Methodology of Pierre Duham.* By ARMAND LOWINGER. Pp. 184. New York: Columbia Univ. Press, 1941. Price, \$2.25.
- Surface Chemistry.* Edited by FORREST RAY MOUTON. Pp. 160; many figs. and tables. Washington, D. C.: Amer. Assn. for the Advancement of Science, 1943. Price, members, \$2.75; to others, \$3.25.
- Rorschach's Test.* I. Basic Processes. By SAMUEL J. BECK, Ph.D., Head of Psychology Laboratory, Department of Neuropsychiatry, Michael Reese Hospital, Chicago; Associate Professor of Psychology, Northwestern Univ. Foreword by WILLARD L. VALENTINE, Ph.D., Head of Dept. of Psychology, Northwestern Univ. Pp. 223; many figs. and tables. New York: Grune & Stratton, 1944. Price, \$3.50.
- Photo-micrography.* By R. M. ALLEN. Pp. 265; 50 plates; 175 figs. New York: D. Van Nostrand, 1941. Price, \$5.50.
- Persistence and Change in Personality Patterns.* By KATHERINE ELIOTT ROBERTS and VIRGINIA VAN DYNE FLEMING. Monographs of the Society for Research in Child Development. Vol. VIII, No. 3 (Serial No. 36). Pp. 206; 26 tables. Washington, D. C.: National Research Council, 1943. Price, \$1.50.
- NEW EDITIONS**
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# THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

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## ORIGINAL ARTICLES

### THIOURACIL STORAGE IN THE THYROID AS AFFECTED BY THYROTROPIC HORMONE AND POTASSIUM IODIDE

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THIOURACIL has recently been used in the treatment of thyrotoxicosis.<sup>1,2</sup> It leads to a fall in the basal oxygen consumption to normal levels with an associated clinical improvement and a decrease in the protein bound iodine content of the plasma. The effectiveness of this drug in inducing the foregoing changes is due to its inhibitory action on the production of the thyroid hormone. Therefore, the amount of thiouracil stored in the thyroid is of importance.

Using the method of Williams, Jandorf and Kay,<sup>4</sup> the content of thiouracil in the thyroid glands of thyrotoxic patients treated with thiouracil has been determined for varying intervals. These values have been compared with those obtained in "normal" glands. The latter values were obtained by administering thiouracil to patients without thyroid disease but with a hopeless prognosis, and estimating the amount of the drug in the thyroid gland at postmortem. However, the variability in the pathologic processes in each group makes it desirable to study this subject under better controlled conditions. Guinea pigs were selected for a continuation of these investigations. These animals were also used for a study of the effect of iodide treatment on the storage of thiouracil in the thyroid.

**Method.** All thiouracil\* was administered as 0.25% solution in the form of its sodium salt at a pH of 8. No other drinking water was given. Thyrotropic hormone\* was injected subcutaneously in daily doses of 0.1 cc., the last

\* We are indebted to the Lederle Laboratories, Inc., Pearl River, N. Y., for the supply of thiouracil and to Parke Davis & Co., Detroit, Mich., for the thyrotropic hormone (Antuitrin T). Each cc. of this hormone contains approximately 50 Junkmann-Schöller units.

injection being given 24 hours before the animal was killed. The guinea pigs receiving iodide therapy were given potassium iodide in the form of an aqueous solution containing 50 mg. per 100 cc. When iodide was given with the thio-uracil, the two substances were given in one solution in the same concentration as above. The animals were killed by means of a blow on the head. The thyroid gland was dissected out, weighed on a microbalance, minced, placed in about an equal volume of 1 N sodium hydroxide and then analyzed for the thio-uracil content according to the method of Williams, Jandorf and Kay.<sup>4</sup>

Five series of animals were studied as follows:

*Series No. 1.* Five guinea pigs weighing from 416 to 555 gm. were given thio-uracil for 6 days. Two of these animals were also injected with thyrotropic hormone for 7 days, beginning 1 day before the thio-uracil treatment.

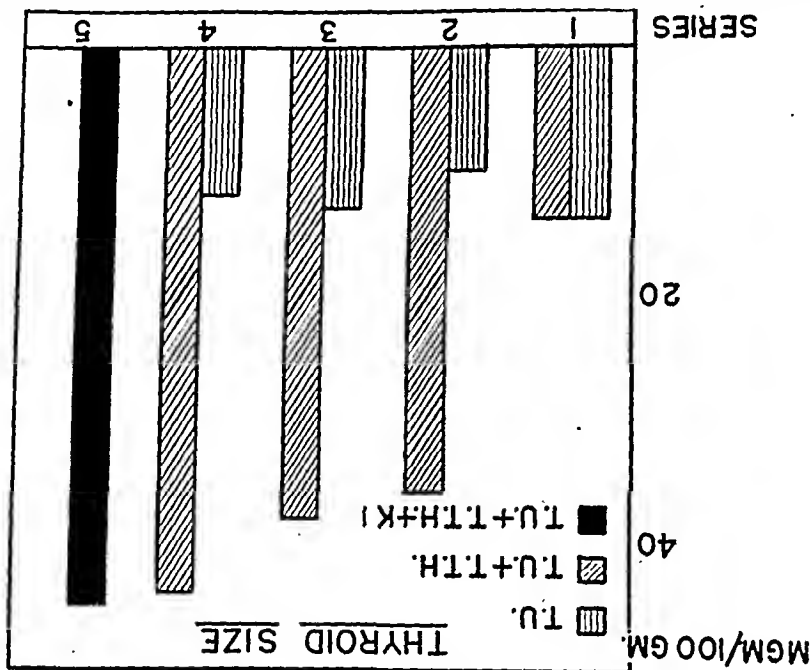


Fig. 1.—Effect of thio-uracil (T.U.), thyrotropic hormone (T.T.H.) and potassium iodide (KI) on the average size of the thyroid expressed in terms of wet weight per 100 gm. body weight. The series numbers are explained in the text.

*Series No. 2.* Six animals, 5 weighing from 414 to 506 gm. and 1 weighing 249 gm., were given thio-uracil for 4 days. Three of these animals were also given thyrotropic hormone for 5 days.

*Series No. 3.* Eleven animals, weighing from 204 to 253 gm., were given thio-uracil for 5 days; 6 were also given thyrotropic hormone for 6 days.

*Series No. 4.* Six guinea pigs, 5 weighing from 321 to 375 gm. and 1 weighing 475 gm., were given thio-uracil for 10 days. Three of these were also given thyrotropic hormone for 11 days.

*Series No. 5.* Six animals, weighing from 300 to 510 gm., were given thio-uracil for 9 days, potassium iodide for 9 days and thio-uracil for 5 days.

In each series the thyrotropic hormone therapy was started 1 day before any other treatment because it was our purpose to have the animals in a hyper-thyroid state when thio-uracil or iodide was given. The experiment dealing with the pigs in Series No. 5 was designed to be somewhat comparable to thyrotoxic patients treated first with iodide and then thio-uracil.

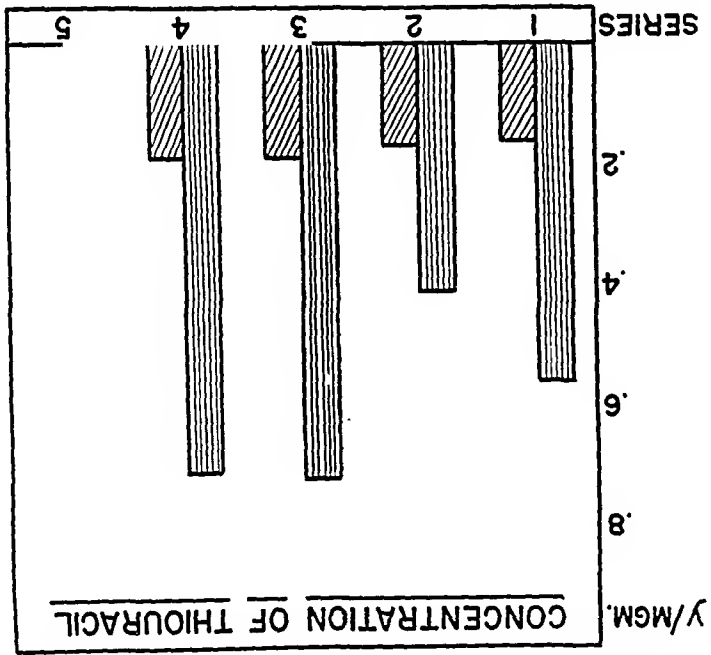


Fig. 2.—Effect of thyrotopin and potassium iodide upon the average concentration of thiouracil in the thyroid gland. Symbols as in Figure 1.

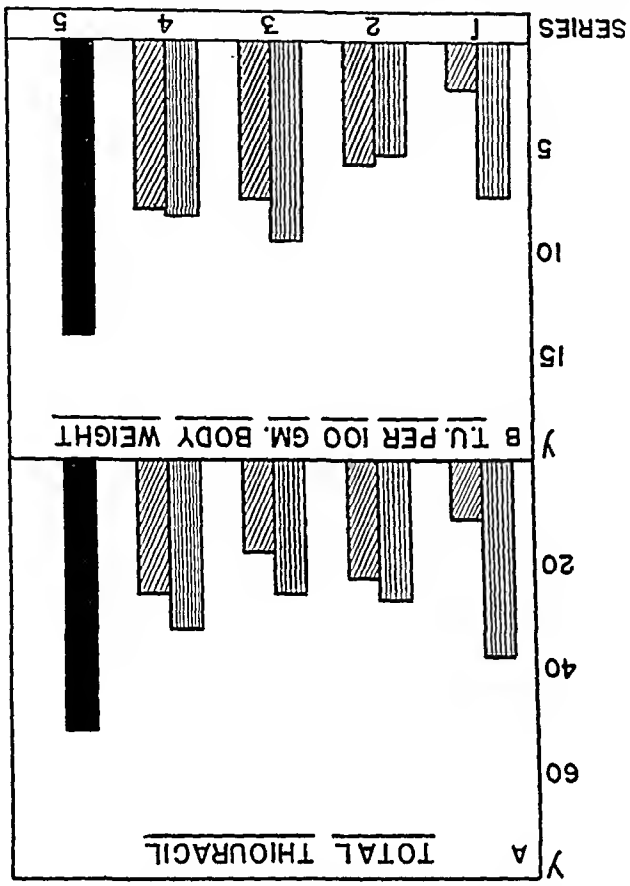


Fig. 3.—A, Average total thiouracil in each thyroid gland. B, Average content of thiouracil in thyroid per 100 gm. of body weight. Symbols as in Figure 1.

**Results.** The guinea pigs receiving thyrotropic hormone as well as thiouracil had much larger thyroid glands than the ones receiving thiouracil alone (Fig. 1). Potassium iodide did not prevent the thyroid enlargement; on the contrary, the glands in this group were the largest of any. These changes are more marked than are observed in the rat. In the rat there is not much difference in the size of the thyroid gland whether the animal is receiving thiouracil alone or in conjunction with the thyrotropic hormone therapy.<sup>4</sup> This is because thiouracil is much more goiterogenic in rats, while thyrotropic hormone is much more goiterogenic in guinea pigs.

Thiouracil was found to be distinctly more concentrated in the thyroid of animals receiving no thyrotropic (thyrotropic hormone) (Fig. 2). Iodide therapy increased the concentration of thiouracil in the guinea pigs treated with thyrotropin. Although the concentration was much greater in the animals receiving thiouracil without thyrotropin, their thyroids were much smaller, and, therefore, one would not expect so great a disparity in the absolute amount of the drug in the thyroid. With this consideration in mind we determined the total amount of thiouracil in the thyroid gland (Fig. 3, A). The animals receiving no thyrotropic hormone were found to have a larger amount in the whole gland than the ones receiving the hormone. However, the pigs given iodide in conjunction with thyrotropin had distinctly more thiouracil in the thyroid than was present in either other group. Since there was some variation in the body weight of the animals we have calculated the amount of thiouracil present on the basis of the body weight. However, essentially the same relative changes were found (Fig. 3, B).

Since the site of action of thiouracil is presumably in the thyroid gland, the concentration attained at this site would seem to be highly important. A consideration of the clinical application of the above observations suggests that patients may require a larger dosage of thiouracil when they are in a hyperthyroid state than when they are not. Our clinical experience also indicates that such is the case. The foregoing experiments also suggest that iodide given in conjunction with thiouracil might possibly enhance the effectiveness of the latter since it increases the storage of thiouracil in the thyroid. At the present time we are investigating this problem clinically.

**Summary.** Thyrotropic hormone given with thiouracil is much more goiterogenic in guinea pigs than is thiouracil alone. Thyrotropin tends to decrease the amount of thiouracil stored in the thyroid while potassium iodide greatly increases the storage of this substance.

**ADDENDUM:** In interpreting the results of our experiments, one must bear in mind the possibility that thiouracil might have formed a combination with iodine either in the drinking water, since both compounds were in one solution, or that these substances might have combined in the thyroid. It is known<sup>5</sup> that iodine reacts with thiouracil to form formamidine disulfide hydriodide, but there is no evidence that this reaction occurs in the body.

In order to study certain biologic and chemical effects of a mixture of thiouracil and iodide we administered these substances in the drinking water to guinea pigs, using 0.25 per cent thiouracil and 50 mg. % potassium iodide. To another group of guinea pigs we gave the same amount of thiouracil but no iodide. At the end of 10 days the



stock solutions contained the same concentration of thiouracil. At this time there was essentially no difference in the 2 groups of animals as concerned the size of the thyroid, the histologic structure, the concentration of thiouracil in the thyroid gland and the total amount of this drug in the thyroid.

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INTERFERENCE BETWEEN INACTIVE AND ACTIVE  
VIRUSES OF INFLUENZA\*I. THE INCIDENTAL OCCURRENCE AND ARTIFICIAL INDUCTION  
OF THE PHENOMENON

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INTERFERENCE of one active virus with the propagation of another in mutually susceptible host cells has been described repeatedly. This phenomenon has been observed first among phytopathogenic viruses.<sup>16,18,24</sup> Although suggestive evidence of its occurrence among animal viruses may be drawn in retrospect from earlier publications, interference was clearly demonstrated first between neurotropic and viscerotropic strains of yellow fever virus by Hoskins<sup>14</sup> and between encephalitogenic and non-encephalitogenic herpes virus by Magrassi and others.<sup>7,19</sup> The phenomenon does occur not only among viruses as closely related as those mentioned—and as additional examples may be cited interference between various strains of poliomyelitis virus<sup>15</sup> and between biologically distinct strains of influenza virus<sup>5</sup>—but interference has been noted also between agents serologically quite unrelated. Thus Findlay and MacCallum<sup>8</sup> have observed interference between the viruses of Rift Valley fever and yellow fever. Dalldorf and Douglas<sup>9</sup> demonstrated a "sparing effect" of lymphocytic choriomeningitis infection in monkeys on superinfection with the virus of poliomyelitis. Similar observations have been made with various serologically unrelated animal viruses and bacteriophages.<sup>2,6,9,17,21,25</sup> This apparent non-specificity, together with the rapidity with which protection by interference could be obtained, set this phenomenon quite apart from immunologic reactions. However, interference has

\* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Children's Hospital of Philadelphia.

been noted only between certain viruses and not between others, perhaps signifying biologic relationship, and examples have been reported of typical inclusions due to two different viruses in a single host cell,<sup>1,22</sup> although in different structures (nucleus and protoplasm). The data obtained in interference of two active agents permit many explanations. One would have to think of hindrance of spread of the second virus infection due to pathologic changes in the host caused by the first. Taylor and Sprunt<sup>23</sup> have shown that increased tissue fluid may hinder the spreading of vaccinia infection in the skin of rabbits. One might consider virucidal properties of either the interfering virus or of abnormal metabolic products of the infected host cell. Greater avidity of one virus for certain essential nutrients or enzyme systems appears as another possible explanation. Finally, a blockade of "cell receptors" has been assumed. Not every one of the suggested explanations can account for all the observed facts, and, on the other hand, it is conceivable that interference may be caused by different mechanisms in the various instances.

A new aspect of the problem was introduced by Luria and Delbrück<sup>17</sup> when they noted that bacteriophage inactivated by ultra-violet irradiation still produced interference not only with a heterologous strain of active virus but also with the homologous agent. Studying this system, the authors concluded that the interfering agents compete for some key material within the host cell, possibly an enzyme. How far these data are applicable to animal viruses remains to be seen. However, it has been shown that irradiated influenza virus interferes with the propagation of the active agent in the allantoic sac of the chick embryo,<sup>11</sup> and Jungelut and Sanders make brief mention of a similar observation with the virus of poliomyelitis in mice, for which experimental data have not yet been published.<sup>15</sup>

It is the aim of this series of publications to study the interference phenomenon produced by inactivated influenza virus from various points of view. The present communication is an extension of the preliminary paper,<sup>11</sup> demonstrating the phenomenon as it occurs incidentally or following artificial inactivation of influenza virus. This study was greatly aided by the recent observation of Hirst,<sup>13</sup> who found that preparations of active and inactivated influenza viruses agglutinate chick red blood cells. This hemagglutination reaction can be utilized for the rapid and quantitative determination of virus multiplication.

**Methods and Materials.** *Virus.* Embryonated eggs at the 10th day of incubation were inoculated by the allantoic route<sup>10</sup> with 0.5 ml. of virus suspension suitably diluted in buffered saline solution of pH 7.0. The PR-8 and WS strains of influenza A and the Lee strain of influenza B virus were mostly employed. After sealing the hole in the shell above the air sac through which the injection was made, the eggs were incubated at 36° to 37° C. for varying periods of time as indicated in the text. Mortality of the embryos at this temperature due to influenza was usually negligible regardless of the concentration of the inoculum. At the end of the incubationary period the eggs were chilled at -10° to -15° C. for 30 to 60 minutes, or at 4° C. for 2 to 4 hours to prevent bleeding during harvest of the allantoic fluids. Each experimental group consisted of at least 8 to 10 eggs, and the fluids harvested from each were

either pooled directly or, in later experiments, samples of individual fluids were kept for separate study and equal amounts of each fluid were pooled. Adequate samples of the various pools were stored immediately after harvest in sealed ampules at  $-70^{\circ}\text{C}$ . until titration for virus activity in mice or chick embryos was possible, and for eventual repetition of doubtful results.

Large batches of allantoic fluid for irradiation or for standard test virus to be kept at  $-70^{\circ}\text{C}$ . were harvested by a suction device (Fig. 1). It consists of a 10 ml. volumetric pipette with closed tip and an opening at its side. The pipette is bent in the manner indicated to permit easier operation, and inserted through a rubber stopper fitting into suitable bottles. One arm of a glass T-piece is inserted through another hole in the stopper, the horizontal arm is connected by light rubber tubing to an aspirator operating at a negative pressure of approximately  $-2\text{ cm. Hg}$ , and the third arm, extending vertically, is left open. The flask is grasped around the neck so that the index finger may conveniently reach the open end of the T-piece. While idling, the air

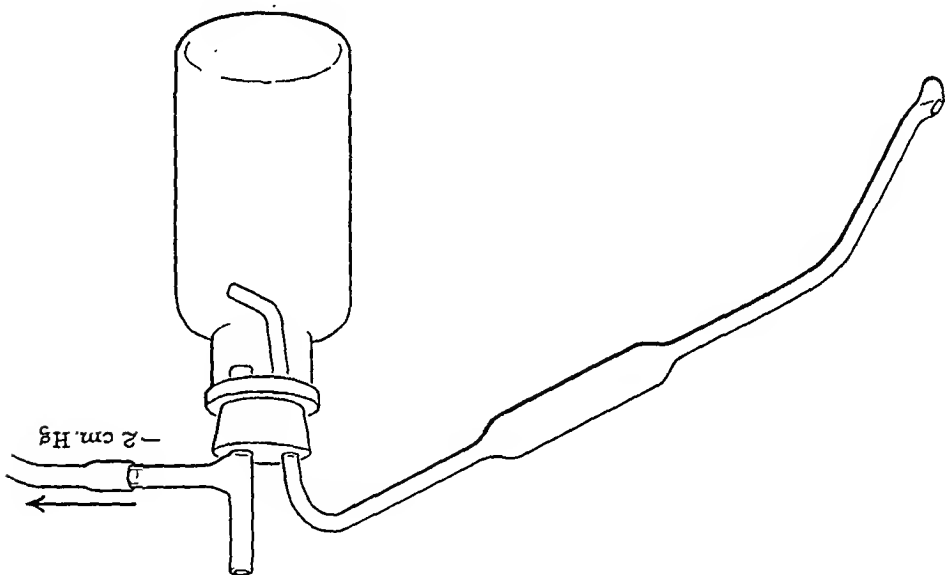


Fig. 1.—Suction device for the harvest of allantoic fluid.

passes from the open end of the T-piece directly to the aspirator without entering the flask. After the tip of the pipette is introduced into the allantoic cavity, its opening is directed towards the inside wall of the egg. The glass drop at the tip is designed to keep tissues away. The free end of the T-piece is closed with the index finger and the allantoic fluid is aspirated into the pipette. The bulb is required to slow down the flow, and in case of rupture of the yolk sac or admixture of blood, release of the index finger will stop suction immediately and the unwanted fluid runs back into the egg. The pipette may be washed with sterile saline solution by repeated aspiration and release before resuming the harvest. Large batches of allantoic fluid (200 to 600 ml.) have been collected with one pipette by this method without bacterial contamination. *Virus Titration in Mice.* Serial ten-fold dilutions of the allantoic fluid were made in sterile broth and 4 to 6 white mice each inoculated intranasally under light ether anesthesia with 0.05 ml. of the different dilutions. The mice were 3 to 4 weeks of age and weighed between 12 and 15 gm. Animals found dead during the experimental period were examined for typical lesions. Surviving mice were sacrificed on the 10th day and the degree of pulmonary involvement recorded as 4 = lungs totally consolidated; 3 =  $\frac{3}{4}$  of lungs consolidated; 2 =  $\frac{2}{4}$  of the lungs; 1 =  $\frac{1}{4}$  of the lungs; 0 = less than  $\frac{1}{4}$  consolidated; and

0 = no visible lesions. The 50% mortality end-point ( $LD_{50}$ ) was calculated according to the method of Reed and Muench.

*Agglutination of Chick Red Cells.* The technique described by Hirst was used.<sup>13</sup> Serial two-fold dilutions of the infected allantoic fluids were made in saline solution and an equal amount (1 ml.) of a 2% suspension of three-washed chick red cells added by an automatic pipette. The degree of sedimentation of red cells was recorded after 75 minutes at room temperature by determining the percentage of red cells left in suspension with the aid of a photo-electric densitometer. The end-point was arbitrarily chosen as the final dilution of allantoic fluid in which enough red cells were left in the supernatant fluid to correspond to a 0.63 to 0.5% suspension. In case the transition from a negative reading to a strongly positive sedimentation occurred from one tube to another, the titer was considered to be halfway between these two dilutions. In all experiments, at least two determinations of the agglutination titer were carried out on two different days with chick cells not older than 3 days. In all cases the average titer was computed and recorded in the tables and figures. The determinations were always found to agree within one step of the dilutions.

*Ultra-violet Irradiation of Virus.* In the early experiments, a Hanovia Examalite quartz lamp was used. A General Electric germicidal lamp of 18 inch length was substituted later. This lamp was installed under a hood and the virus was placed in open Petri dishes containing 15 to 18 ml. of allantoic fluid each on a tray at a distance of 7 inches from the lamp. The intensity of irradiation at this distance amounted to approximately  $275 \frac{W}{cm^2}$ . Mechanical rocking of the tray in an up-and-down movement of about 1 inch excursion 90 times a minute kept the allantoic fluid agitated in such a way as to avoid stationary waves or formation of foam.

*Experimental. The Incidental Occurrence of Interference.* In passing strains of influenza virus by the allantoic route, it was noted that frequently a concentrated inoculum would give results distinctly inferior to those obtained by injection of more dilute infected allantoic fluid. This point is demonstrated in Table 1, where hemagglutinin titers of pools of 8 to 10 allantoic fluids derived from subcultures in various dilutions of the PR-8 and WS strains of influenza A and the Lee strain of influenza B virus are compared. All three strains show this phenomenon to some extent, although not regularly. Three additional strains of influenza A virus, not represented in the table, have yielded similar results. This inhibitory effect is particularly marked with the Lee strain of influenza B virus, in which case sometimes no or only low agglutination titers were recorded in allantoic fluids derived from concentrated inocula, while more dilute seed produced the usual high titers. As will be shown later, the low titers encountered in subcultures of undiluted infected allantoic fluid may be due to residual hemagglutinin from the inoculation rather than the result of virus propagation. In the experiments cited in Table 1, all fluids of one series were harvested after the same period of incubation, usually after 48 hours. It was conceivable that fluids derived from concentrated inocula may have had a higher hemagglutinin titer at an earlier stage of the incubation period, with subsequent loss of activity on further incubation. However, this could not be verified as inspection of Figure 2 will show. The figure summarizes two experiments conducted either with influenza A (PR-8) or with B virus (Lee). When the allantoic fluids of groups of eggs inoculated with different concentrations of virus

were collected after various times of incubation, it was found that the agglutinative titers of allantoic fluids derived from concentrated inocula were lower throughout the series of fluids than those obtained from fluids following injection of dilute virus. While the agglutinative titer after reaching its maximum remains more or less constant for several days of incubation, the infectivity of the allantoic fluids as measured by inoculation of mice falls off after attaining its peak. Similar observations have been made by Burnet with the Melbourne strain of influenza A.<sup>4</sup> The height of this peak, like that of the hemagglutinin titer, depends on the concentration of the inoculum, and under optimal conditions 100 to 300 times more active virus was obtained from dilute inocula than from concentrated ones. Maximal concentration of active virus and hemagglutinins for the various conditions was reached earlier following injections of more concentrated virus, and correspondingly later when dilute virus was employed. It is to be noted that the peak of active virus is reached earlier than the maximal hemagglutinin titer.

TABLE 1.—RELATION BETWEEN CONCENTRATION OF SEED CULTURE AND TITER OF HEMAGGLUTININS IN SUBCULTURE

Derivation of seed culture		Hemaggl. titer of subculture inoculated with seed culture in dilution	
Prepared from dilution	Time of incubation (hours)	10 <sup>-1</sup>	10 <sup>-2</sup>
PR-8	10 <sup>-5</sup>	48	620
	10 <sup>-4</sup>	24	256
	10 <sup>-3</sup>	96	192
	10 <sup>-2</sup>	96	128
	10 <sup>-1</sup>	96	80
WS	10 <sup>-1</sup>	48	192
	10 <sup>-1</sup>	48	128
	10 <sup>-1</sup>	48	80
	10 <sup>-1</sup>	48	128
	10 <sup>-1</sup>	48	128
Lee	10 <sup>-1</sup>	24	96
	10 <sup>-1</sup>	96	144
	10 <sup>-1</sup>	96	32
	10 <sup>-1</sup>	96	128
	10 <sup>-1</sup>	96	64
n.t. = not tested.	10 <sup>-1</sup>	48 + 72	16
	10 <sup>-1</sup>	96	3
	10 <sup>-1</sup>	96	12
	10 <sup>-1</sup>	96	48
	10 <sup>-1</sup>	96	16

*Artificial Production of Interference.* Experiments on the injection of eggs with virus partially inactivated either by heating to 56° C. or by irradiation with ultra-violet light are summarized in Table 2. The PR-8 virus in allantoic fluid was heated at 56° C. in individual test tubes, and one tube each removed from the water bath at the indicated time. The original infected allantoic fluid had no inhibitory effect. After five minutes of incubation at 56° C., formation of hemagglutinins following injection of undiluted fluid reached a titer of only 1:32, while 1000-fold diluted seed produced a titer of 1:1024. Heating for 10

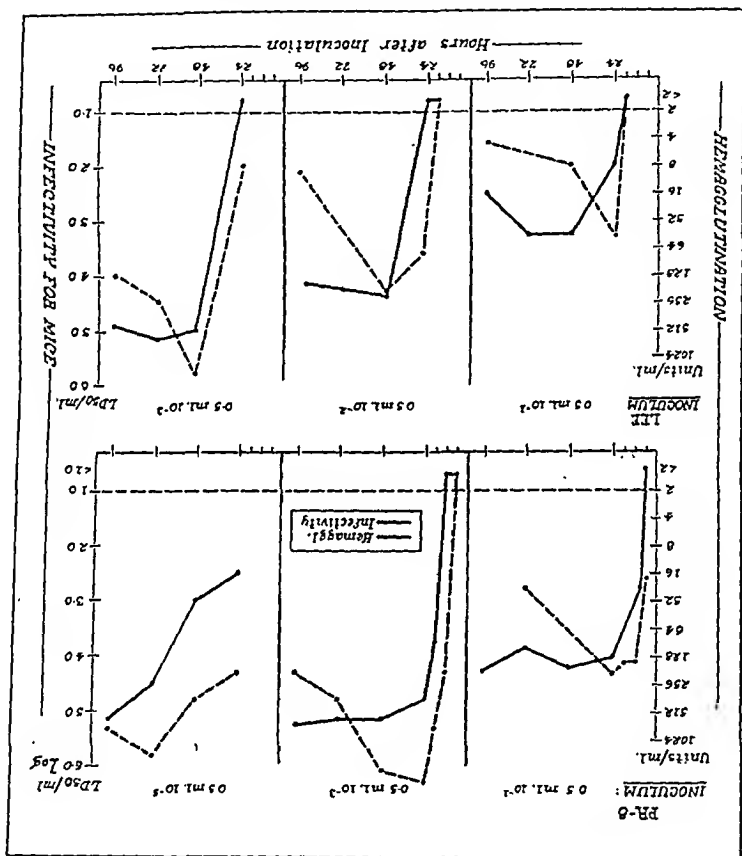


Fig. 2.—Development of active virus and hemagglutinins following inoculation of various concentrations of seed culture.

minutes produced even more striking results in that the undiluted inoculum produced no measurable hemagglutinins while a 100-fold diluted inoculum of the same heated fluid gave a high titer. Similar results were obtained after ultra-violet irradiation of the WS strain of influenza A virus. The non-irradiated preparation showed some inhibition, but the effect could be markedly increased by irradiation for 2 minutes, and with 10 minutes' irradiation the results were very similar to those obtained with the PR-8 strain by heating to 56° C. for 10 minutes. It is to be noted that the hemagglutinin titer of the inoculum was definitely affected by heating, since it decreased from

1:512 in the original fluid to 1:192 after 30 minutes at 56° C., and after 60 minutes frequently only 20% or less of the original titer was left. The ultra-violet irradiation of the allantoic fluid did not have this effect under the conditions of the experiment, and the titer stayed constant for the duration of the exposure. Different results were obtained after irradiation of dialyzed allantoic fluids or with exposure to stronger irradiation, as will be shown later.

TABLE 2.—EFFECT OF HEAT AND ULTRA-VIOLET IRRADIATION ON INHIBITORY PROPERTY OF CONCENTRATED INOCULA

Inoculum		Time of inactivation (min.)		Hemagglutination titer		Hemagglutinin titer of subculture inoculated in dilution	
Strain of virus	Means of inactivation	Heat (56° C.)	Ultra-violet irradiation	WS	PR-8	WS	PR-8
				30	30	10 <sup>-1</sup>	10 <sup>-1</sup>
				20	20	10 <sup>-2</sup>	10 <sup>-2</sup>
				10	10	10 <sup>-3</sup>	10 <sup>-3</sup>
				5	5	10 <sup>-4</sup>	10 <sup>-4</sup>
				2	2	10 <sup>-5</sup>	10 <sup>-5</sup>
				512	512	10 <sup>-6</sup>	10 <sup>-6</sup>
				512	512	10 <sup>-7</sup>	10 <sup>-7</sup>
				512	512	10 <sup>-8</sup>	10 <sup>-8</sup>
				512	512	10 <sup>-9</sup>	10 <sup>-9</sup>
				512	512	10 <sup>-10</sup>	10 <sup>-10</sup>
				512	512	10 <sup>-11</sup>	10 <sup>-11</sup>
				512	512	10 <sup>-12</sup>	10 <sup>-12</sup>
				512	512	10 <sup>-13</sup>	10 <sup>-13</sup>
				512	512	10 <sup>-14</sup>	10 <sup>-14</sup>
				512	512	10 <sup>-15</sup>	10 <sup>-15</sup>
				512	512	10 <sup>-16</sup>	10 <sup>-16</sup>
				512	512	10 <sup>-17</sup>	10 <sup>-17</sup>
				512	512	10 <sup>-18</sup>	10 <sup>-18</sup>
				512	512	10 <sup>-19</sup>	10 <sup>-19</sup>
				512	512	10 <sup>-20</sup>	10 <sup>-20</sup>
				512	512	10 <sup>-21</sup>	10 <sup>-21</sup>
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				512	512	10 <sup>-26</sup>	10 <sup>-26</sup>
				512	512	10 <sup>-27</sup>	10 <sup>-27</sup>
				512	512	10 <sup>-28</sup>	10 <sup>-28</sup>
				512	512	10 <sup>-29</sup>	10 <sup>-29</sup>
				512	512	10 <sup>-30</sup>	10 <sup>-30</sup>
				512	512	10 <sup>-31</sup>	10 <sup>-31</sup>
				512	512	10 <sup>-32</sup>	10 <sup>-32</sup>
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				512	512	10 <sup>-40</sup>	10 <sup>-40</sup>
				512	512	10 <sup>-41</sup>	10 <sup>-41</sup>
				512	512	10 <sup>-42</sup>	10 <sup>-42</sup>
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				512	512	10 <sup>-95</sup>	10 <sup>-95</sup>
				512	512	10 <sup>-96</sup>	10 <sup>-96</sup>
				512	512	10 <sup>-97</sup>	10 <sup>-97</sup>
				512	512	10 <sup>-98</sup>	10 <sup>-98</sup>
				512	512	10 <sup>-99</sup>	10 <sup>-99</sup>
				512	512	10 <sup>-100</sup>	10 <sup>-100</sup>

\* One or 2 out of 6 allantoic fluids positive.  
n.t. = not tested.

The experiments reported thus far indicated that inactive virus accumulating under certain conditions in the allantoic fluid, or artificially increased by heating or irradiation of the infectious materials, when injected into the allantoic cavity of the chick embryo, interfered with the propagation of the active agent present in the same preparation. Thus, protection of susceptible cells was obtained rapidly, and the possible value of this phenomenon in prevention of influenza infections suggested further investigations. It was obvious that the foregoing set-up did not permit quantitative estimations and analysis of the relationships between active and inactivated virus. Attempts were made, therefore, to produce on the one hand fully inactivated virus with the interfering property largely intact, and to test for the interfering property with known amounts of the active agent. The following experiments were designed to lead towards this goal.

Infected allantoic fluids irradiated for as long as 60 minutes were usually not completely inactive. Although a single subculture of such fluids in mice or chick embryos frequently did not disclose signs of infection, a second passage often did. By these means it was shown that allantoic fluid from the first passage contained, on occasion, as much as 10,000 L.D<sub>50</sub> for mice per milliliter, without having revealed agglutination of chick red cells. In other instances only very little virus was demonstrable by second passage. In spite of this incomplete inactivation of virus, experiments were conducted to test whether injection of active virus either at the time of or following the inoculation of irradiated fluids would prevent the propagation of the virus. The results of some of these experiments are summarized in Table 3. In all instances groups of eggs were inoculated with 0.5 ml. amounts of

either irradiated infected or normal allantoic fluid. Those injected with the infected material were subsequently tested with either active virus or saline solution (0.2 ml.), while those receiving normal fluid were tested only with virus. After further incubation of 48 hours, the allantoic fluids were collected and tested for agglutination of chick red cells and infectivity for mice. As can be seen in the table, inoculation of irradiated virus into the allantoic cavity usually did not of itself produce measurable amounts of hemagglutinin. Secondary infection of active virus into eggs primarily inoculated with irradiated virus did not alter the results, and no agglutination of red cells was obtained with the allantoic fluids harvested from these eggs. Titration of these fluids in mice, however, revealed measurable amounts of active virus, regardless of whether the eggs were secondarily injected with active virus or saline, and the  $LD_{50}$  frequently did not differ significantly between the two groups. These titers, however, were markedly lower than those obtained with fluids derived from eggs tested with active virus following injection of normal fluid. In this latter case the fluids contained as much as 1000 to 10,000 times the amount of active virus in addition to high hemagglutinin titers. These results did not differ very much, regardless of whether the test virus was injected simultaneously with, or 3 to 24 hours following the injection of the irradiated material. Only homologous interference reactions are shown in Table 3. Cross-interference has been noted between various strains of influenza A, and between influenza A, B, and swine virus. These tests will be discussed in a subsequent paper.

TABLE 3.—SUMMARY OF INTERFERENCE EXPERIMENTS WITH NON-DILUTED ALLANTOIC FLUIDS IRRADIATED WITH ULTRA-VIOLET LIGHT

Expert- ment	1.	First injection			Second injection		
		Time of ultra-violet irrad. (min.)	Strain of virus	Time of infectiv- ity for injection (hours)	Time after last injection (hours)	Saline solution	Active virus
		30	WS (24 hr.) *	30	0	Hemag- glutinin in mice	$LD_{50}$ Hemag- glutinin in mice
		30	WS (96 hr.)	30	0	Hemag- glutinin in mice	$LD_{50}$ Hemag- glutinin in mice
		30	Normal all. fluid	30	0	Hemag- glutinin in mice	$LD_{50}$ Hemag- glutinin in mice
2.		30	WS (96 hr.)	n.t.	3	10:1	3
		..	Normal all. fluid	n.t.	24	10:1	256
		..	WS (72 hr.)	n.t.	3	<2	<2
3.		60	Normal all. fluid	000000	3	<2	<2
		60	WS (96 hr.)	000000	3	..	384
		30	Lee (96 hr.)	n.t.	0	n.t.	8
4.		30	Normal all. fluid	..	0	n.t.	288

\* (24 hr.) = incubation period of virus used for irradiation.

It could be shown that the hemagglutinin formation was not simply delayed by the injection of irradiated virus previous to active virus, since the results were similar whether the allantoic fluids were harvested 48 or 96 hours after the test virus injection (see Table 4).



The table also shows that the results in individual eggs are usually quite uniform. In the experiment cited, only 3 out of 16 allantoic fluids of the experimental group showed a minimal amount of hemagglutinins. Of the 8 control eggs, all showed strong agglutination of chick red cells in dilution of 1:200, and the pool of these fluids gave a titer of 1:512. This uniformity, however, did not always occur. On occasion, one or the other of the saline controls showed a fairly high titer of hemagglutinins, while the remainder revealed no measurable quantities. These irregularities have not been satisfactorily explained. In other instances 1 or 2 out of 10 eggs tested with active virus following irradiated virus showed a high titer, while the others, as well as the controls tested with saline solution, did not. It is considered probable that the injection of irradiated virus in these cases may not have entered the allantoic cavity at all, or only partly, thereby preventing or reducing interfering activity. Because these failures occurred, it was necessary in all experiments of this type to harvest the allantoic fluids individually and to pool equal amounts of each group for testing. This made it possible to study the regularity of the interference phenomenon and to check back in case of irregularities.

TABLE 4.—RESULTS OF INTERFERENCE AFTER 48 AND 96 HOURS OF INCUBATION

Hemagglutinin titer following second injection of active virus									
Titer of pooled allantoic fluids	Number of chick embryo	Dilution of allantoic fluid	Time of harvest (hours)	Irradiated virus	96	48	Irradiated normal allantoic fluid	+ + indicates more than 63% of red cells sedimented.	+ + indicates more than 63% of red cells sedimented.
<3	1	1:2	48	+	+	+	+	+	+
	2	1:20	96	+	+	+	+	+	+
<2	3	1:20	96	+	+	+	+	+	+
	4	1:20	96	+	+	+	+	+	+
8	5	1:20	96	+	+	+	+	+	+
	6	1:20	96	+	+	+	+	+	+
7	7	1:20	96	+	+	+	+	+	+
	8	1:20	96	+	+	+	+	+	+
512	9	1:20	96	+	+	+	+	+	+
	10	1:20	96	+	+	+	+	+	+
37	11	1:20	96	+	+	+	+	+	+
	12	1:20	96	+	+	+	+	+	+

*Effect of Dialysis of Allantoic Fluid on Inactivation of Virus by Irradiation.* Although these results further confirmed the occurrence of interference between active and inactive influenza virus, the value of the tests was diminished by the presence of relatively large amounts of active virus both in the experimental and control groups. This was due to incomplete inactivation of the agent in straight allantoic fluid by ultra-violet irradiation. An improvement was noted when allantoic fluids were dialyzed against buffered saline solution before exposure to the ultra-violet rays. Since the fluids contain relatively large and variable amounts of urates which absorb ultra-violet light extensively, the removal, or partial removal of these substances by dialysis improved the effectiveness of irradiation. Consequently, when infected allantoic fluid was dialyzed at 4° C. against buffered saline solution of pH 7 for 24 to 48 hours and subsequently exposed to irradiation, it was found that periods of exposure necessary to obtain marked interference with non-dialyzed fluids were far too long for the dialyzed preparations. Under these circumstances the interfering property was destroyed, and the ability to agglutinate chick red cells was reduced

or lost. Whereas non-dialyzed fluids after 30 minutes or more of irradiation still produced influenza lesions in mice, in the case of dialyzed fluids no pulmonary involvement was noted after inoculation of material irradiated for only 5 to 10 minutes. In non-dialyzed fluids the interfering property could be demonstrated after irradiation for 30 to 60 minutes, whereas dialyzed preparations interfered only when exposure to ultra-violet light was shorter than 30 minutes. With longer exposure the interference diminished rapidly and the test virus grew out (positive red cell agglutination), while controls injected with saline solution instead of with virus gave negative tests (Table 5). This shows that the interfering property is not dialyzable and experiments in which infected allantoic fluid was dialyzed after irradiation confirmed this point. The interfering property of allantoic fluid dialyzed after irradiation for 30 to 60 minutes was left intact. Experiments pertaining to this point are included in Table 5.

TABLE 5.—EFFECT OF DIALYSIS ON INTERFERING PROPERTY

Exp.	Strain of virus in allantoic fluid	Dialysis		Time of irradiation (min.)	Hemaggl. units per ml.	Infectivity for mice	Hemaggl. titer in allantoic fluid 48 hours following injection of	
		In relation to irradiation	Ratio				Saline solution virus	Active virus
1.	WS	+	Before	60	1024	000000	<2	<2
	Normal	+	Before	60	<2	000000	<2	320
		-	..	60	<2	000000	..	384
2.	WS:	-	..	30	— 384	22100	<2	<2
		+	Before	60	384	000000	<2	<2
		+	Before	10	256	000000	<2	<2
		+	Before	30	256	000000	<2	64
		+	Before	60	320	000000	<2	384
		+	Before	5	256	000000	<2	<2
		+	Before	10	224	000000	<2	16
		+	Before	30	192	000000	<2	512
		+	Before	60	64	000000	<2	512
		+	After	30	320	0000	<2	<2
		+	After	60	384	000000	<2	<2
	Normal	-	..	30	<2	000000	..	384
3.	PR-8	-	After	60	512	D <sub>41</sub> 41	20	20
		+	After	60	512	D <sub>41</sub> 40	96	64
	Normal	-	..	60	<2	0000	..	512

It was expected, and it is shown in Table 5, that the ratio of volumes between dialyzing fluid and virus preparation was of importance. The more complete the dialysis, the shorter the period of irradiation in which marked interference may be obtained. If the ratio of buffered saline solution to allantoic fluid was 20:1, irradiation for 10 minutes still gave preparations preventing the formation of measurable amounts of hemagglutinin, while with a ratio of 80:1 this was no longer the case. Therefore, in order to have a sufficiently wide range for operation, all fluids for interference experiments were dialyzed against 20 parts of buffered saline solution.

When allantoic fluids were dialyzed in this manner and exposed to ultra-violet irradiation for periods ranging from 1 to 60 minutes, the useful period of exposure, as far as inhibition of hemagglutinin formation was concerned, was found to lie between 1 and 10 minutes, on occasion up to 20 minutes. Table 6 represents a summary of some of the results. Virus irradiated for as little as 1 minute and up to 20

minutes may cause marked inhibition of hemagglutinin formation when injected 3 hours prior to an active test virus, which in control eggs produced high titers. The results appear to be more clear-cut with the WS strain, as no measurable agglutination was found under these conditions, and all fluids from control eggs injected with the irradiated material and saline solution were negative. The PR-8 strain behaved differently, in that low agglutinin titers were found both in control eggs secondarily injected with saline solution and in experimental groups injected with test virus. The titers were below 1:10 in Experiments 5 to 7, when 0.5 ml. amounts of irradiated virus were injected, while higher titers were noted in Experiment 8, in which 1 ml. was given. Only Experiment 4 revealed negative allantoic fluids in both groups when an irradiated fluid of low original red cell agglutinative titer (24-hour-harvest) was used, or when the irradiated virus was diluted nine-fold (Experiment 8b). These observations suggested strongly that some hemagglutinin was left free in the allantoic cavity from the injection of irradiated virus, which was not absorbed and could be recovered on harvest 48 hours after the secondary injection of either test virus or saline solution. More evidence for this point will be presented in the subsequent paper.<sup>12</sup>

TABLE 6.—RESULTS OF INTERFERENCE AFTER VARYING TIMES OF IRRADIATION OF DILYZED INFECTED AFTER ALLANTOIC FLUID

Exp. No.	First injection		Second injection 3 hours later		Hemaggl. titer 48 hours following 2nd injection	
	Strain	of virus	Original hemaggl. titer	Saline	Length of irradiation of 1st inoculum (min.)	
1.	WS	384	<2	112	0	1
	Normal	<2	Virus	..	3	5
2.	WS	384	<2	80	..	1
	Normal	<2	Virus	..	3	5
3.	WS	512	<2	32	..	3
	Normal	<2	Virus	..	3	5
4.	PR-8	160	<2	256	..	3
	Normal	<2	Virus	..	3	5
5.	PR-8	470	<2	512	7	4
	Normal	<2	Virus	..	3	5
6.	PR-8	490	<2	600	..	3
	Normal	<2	Virus	..	3	5
7.	PR-8	256	<2	200	..	3
	Normal	<2	Virus	..	3	5
8.	PR-8	512	<2	..	..	3
	Same	..	..	..	..	3
	Normal	<2	Virus	..	..	3

\* Two out of 10 allantoic fluids positive.

Another fact should be pointed out here. Experiments 4, 5, and 8b in Table 6 show that allantoic fluids from control eggs inoculated with saline solution following the administration of virus irradiated for 10 minutes were negative in the agglutination test. When the fluids were irradiated for 20 minutes some virus apparently grew out, since

some hemagglutinins were found, and this could be confirmed by infection of mice. In other experiments the presence of active virus in the irradiated material could be demonstrated only by certain procedures to be outlined in paper II of the series and finally in a number of instances no virus was found but the interfering property was largely intact. In any event, the amount of active virus was so small that the technique of inactivation by ultra-violet irradiation following dialysis was adopted for the present for the study of the interference phenomenon until safer methods for the complete inactivation of virus have been devised which will leave most of the interfering property intact.

**Discussion.** The observations reported indicated that inactive virus accumulated in the allantoic cavity of the chick embryo infected with influenza A or B, in addition to the active agent. Depending on the relative concentration of the inactive virus, it caused difficulties in passing certain strains of influenza virus by interfering with its propagation. This was notably the case with the Lee strain of influenza B. If one obtained insufficient growth in the subculture (low hemagglutinin titer) it would have appeared correct to use a more concentrated inoculum for subsequent passages, while from the above data the opposite course appears more likely to lead to the desired effect.

The data indicate that correlation between the content of active virus in allantoic fluid and the hemagglutinin titer may exist possibly only during the stage of rapid increase of the active virus, but not after the active virus titer has reached its peak and started to decrease. It is not known, however, whether this process of inactivation is concomitant with the propagation of the active agent.

**Summary.** In passing allantoic fluid infected with the virus of influenza A or B to chick embryos by the allantoic route, it was found that concentrated inocula frequently produced less virus than more dilute ones. This inhibition was found to be due to an accumulation of inactive virus in the allantoic fluid interfering with the propagation of the active agent on subculture. Correspondingly, artificial inactivation of the virus by heating to 56° C. or ultra-violet irradiation for short periods of time increased this inhibitory effect.

In order to study this phenomenon more accurately, attempts were made to obtain, on the one hand, non-infectious virus with the interfering property largely intact; and to test, on the other hand, the inter-

ference by the inactive virus with a known amount of the active agent. Ultra-violet irradiation of dialyzed allantoic fluid gave interfering preparations which frequently fulfilled the stated requirements. Primary injection of such irradiated virus into the allantoic sac of the chick embryos, followed by inoculation of active virus within periods of up to 24 hours, prevented the propagation of the active agent.

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## INTERFERENCE BETWEEN INACTIVE AND ACTIVE VIRUSES OF INFLUENZA\*

### II. FACTORS INFLUENCING THE PHENOMENON

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In the preceding paper it has been shown that influenza virus inactivated under various conditions may interfere with the propagation of the active agent in the allantoic cavity of the chick embryo. This work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Children's Hospital of Philadelphia.

type of interfering phenomenon deserved further attention in view of the fact that it induced rapid protection of susceptible cells. As has been shown, the results of interference did not differ markedly, regardless of whether the irradiated virus was injected 24 hours previous to, or simultaneously with the active test virus. In the present paper, various aspects and factors influencing the phenomenon are analyzed, particularly in regard to prevention of formation of measurable amounts of hemagglutinins following the inoculation of active virus. Problems encountered in the inactivation of virus by irradiation and evidence of the survival of test virus in the allantoic cavity for periods of 24 to 72 hours are presented.

**Methods and Materials.** The methods used for the propagation of the influenza viruses in the allantoic sac, the techniques employed for the determination of infectivity for mice and chick embryos, and for the assay of hemagglutinins have been described in the preceding paper, as well as the apparatus for ultra-violet irradiation.<sup>2</sup>

**Interference Test.** Infected allantoic fluids harvested under conditions minimizing bacterial contamination were dialyzed at 4° C. against 20 parts of buffered saline solution of pH 7 for 40 to 48 hours with repeated stirring of the dialyzing fluid. The dialyzed preparations were stored in suitable volumes in the frozen state at -10° to -15° C. for periods of several months without apparent loss of interfering properties. After thawing, the fluids were left at room temperature for at least 30 minutes and centrifuged at 2000 r.p.m. for 30 minutes to remove precipitated matter. The sediment removed was relatively small since the fluids had been dialyzed. Between 15 and 18 ml. amounts of the allantoic fluids were irradiated in open Petri dishes, usually for 3 to 5 minutes, at a distance of 7 inches from a General Electric germicidal lamp (275  $\mu$  W/cm<sup>2</sup>) under continuous mechanical shaking. The irradiated and bacteriologically sterile fluids may be kept in the refrigerator for several weeks without loss of the interfering quality. For the experiments recorded in this paper, however, the fluids were thawed and inactivated freshly for each test. Normal allantoic fluid was collected and treated in the same manner. In earlier tests, normal allantoic fluid was used which had been harvested from batches of eggs comparable to those from which the interfering fluids were prepared and after the same total period of incubation. This precaution, however, was found to be unnecessary since the age of the embryo from which the normal allantoic fluid was obtained did not affect the results of the propagation of the test virus. Accordingly, any normal allantoic fluid may be used for the control injection.

For the test, 1 ml. of the irradiated infected or normal allantoic fluid was injected by the allantoic route, followed 14 to 18 hours later by 0.2 ml. of suitably diluted active virus or of saline solution. This increase in interval between the two injections, in contrast to the maximum of 3 hours in earlier experiments, reduced the incidence of non-specific deaths of embryos. After further incubation of the eggs at 36° to 37° C. for usually 48 hours, the allantoic fluids were harvested individually, and equal amounts of the corresponding liquids pooled. The undiluted individual allantoic fluids were usually tested qualitatively for the presence or absence of hemagglutinins. The pools were titrated for hemagglutinins and infectivity by methods previously described.<sup>2</sup> Individual fluids were similarly assayed when indicated. Under the conditions described, results of interference experiments were repeatable either when the same starting material was used or when allantoic fluids harvested from eggs under apparently identical conditions were employed.

**Experimental. Quantitative Determination of Interfering Property.** In order to determine the relative amount of interfering agent in the

allantoic fluid, embryos were injected by the allantoic route with various dilutions of irradiated virus and injected 18 hours later either with active virus or saline solution. A control group of eggs injected with normal allantoic fluid followed by active virus served as indicator of the degree of inhibition. As can be seen in Table 1, irradiated PR-8 virus fluids diluted 9 to 81-fold were able to prevent the formation of measurable quantities of hemagglutinin by the test injection of virus. When undiluted or three-fold diluted irradiated virus was injected, hemagglutinins introduced with this injection could be demonstrated when the allantoic fluids were harvested 48 hours after the test injection of virus or saline solution. Dilutions of the irradiated PR-8 virus higher than 1:81 did not prevent the propagation of the test virus as seen by the formation of measurable amounts of hemagglutinins, but even in the case of dilutions of 1:243 or 1:729, the hemagglutinin titer frequently did not equal that of the control allantoic fluids collected from eggs first injected with normal fluid and later with active virus. The activity of the irradiated WS virus preparations usually was weaker, and little or no residual hemagglutinin was from the first injection were found at the termination of the tests, while the results with the Lee strain of influenza B were comparable to those obtained with PR-8 virus. Experiment 5 of Table 1 shows an exception to the other experiments recorded in this series, discussion of which will be postponed until a later section of this paper.

TABLE 1.—TITRATION OF INTERFERING PROPERTY

Exp. No.	Strain of virus	First injection		Second injection		Hemagglutinin titer 48 hours after second inoculation following injection of									
		Irradiated virus		18 hrs. later		Irradiated virus in dilution									
1.	WS	0000	0000	Saline	Und.	1:3	1:9	1:27	1:81	1:243	1:729	Normal allantoic fluid und.			
	(72 hr.) *			Virus	4	<2	<2	64	512	768	512	<2			
2.	WS	00000	<2	Saline	<2	112	320	512	..	..	..	..			
3.	PR-8	0000	32	Saline	48	3	<2	<2	<2	<2	<2	<2			
4.	PR-8	000	16	Saline	64	3	<2	<2	<2	<2	<2	<2			
5.	PR-8	..	14	Saline	7	<2	<2	<2	40	32	64	..			
6.	PR-8	..	28	Saline	12	..	<2	<2	4	128	<2	..			
7.	PR-8	..	24	Saline	16	3	<2	<2	<2	<2	<2	<2			
8.	PR-8	00000	48	Saline	32	3	<2	<2	<2	<2	<2	<2			
9.	Lee	0000	24	Saline	16	3	<2	<2	<2	64	384	..			
10.	Lee	0000	16	Saline	24	3	<2	<2	<2	<2	<2	..			
	(72 hr.)			Virus	..	..	<2	<2	<2	4	192	768			

\* (72 hr.) = the incubation period of virus used for irradiation.

It was considered that the concentration of active virus in the test dose used for demonstrating the interfering property of a given irradiated fluid might conceivably influence the results. Table 2 shows an

from smaller doses.

Hemagglutinin titer 48 hours after test inoculation following injection of

Number of ID <sub>50</sub> in test virus injection		Irradiated virus in dilution		Normal - allantoic fluid und.	
Saline		1:3	1:9	1:27	1:243
3 × 10 <sup>6</sup>	7	<2	<2	<2	128
3 × 10 <sup>5</sup>	4	<2	<2	<2	256
3 × 10 <sup>4</sup>	3	<2	<2	<2	128
3 × 10 <sup>3</sup>	3	<2	<2	<2	384
3 × 10 <sup>2</sup>	7	<2	<2	<2	384
3 × 10 <sup>1</sup>	3	<2	<2	<2	288
3 × 10 <sup>0</sup>	8	<2	<2	<2	320
Saline	3	<2	<2	<2	512

*Influence on Interference of Incubationary Period of Virus Used for Irradiation.* In earlier experiments the impression was gained that the interfering property of irradiated virus was particularly pronounced in allantoic fluids harvested after extended periods of incubation, *i. e.*, fluids collected 72 to 96 hours after inoculation, appeared to be more suitable than fluids harvested after 24 to 48 hours. When this was checked with the present technique it could not be verified. Several hundred 10-day-old embryos were inoculated by the allantoic route with a suitable dilution of PR-8 virus and comparable groups of eggs were harvested after 24, 48, 72, and 96 hours of incubation. The respective allantoic fluids were dialyzed together against 20 parts of buffered saline for 40 hours. When these fluids were irradiated for 90 seconds and then compared with each other in interference tests, no great differences could be noted. The results of the agglutination test are shown graphically in Figure 1. All fluids in dilution 1:81 prevented the formation of hemagglutinins following the test virus injection. A dilution of 1:243 and 1:729 gave only limited reductions in titer as compared with the controls. Longer irradiation of the fluids (3 or 5



minutes) destroyed some of the interfering property of fluids harvested after 24 hours and 48 hours, but the results of the 72- and 96-hour fluids were not markedly different from the results obtained after irradiation for 90 seconds. Longer periods of irradiation, as shown previously, may destroy the interfering property also in these fluids. The relative constancy of interference by the various fluids were somewhat unexpected, in view of the differences in their hemagglutinin titer. It was only one-third as high in the 24-hour fluid as in the 48- and 72-hour preparations, while the 96-hour material was lower again probably because of the removal of larger amounts of precipitated matter following freezing and thawing. The hemagglutinin titers were, in order of the collection of the fluids, 1:160; 1:470; 1:490; and 1:356. Determinations of the infectivity of the allantoic fluid immediately after harvest showed that the fluids contained  $10^{5.3}$ ,  $10^{5.5}$ ,  $10^{6.1}$  and  $10^{6.3}$  50%-mortality doses for mice per milliliter.

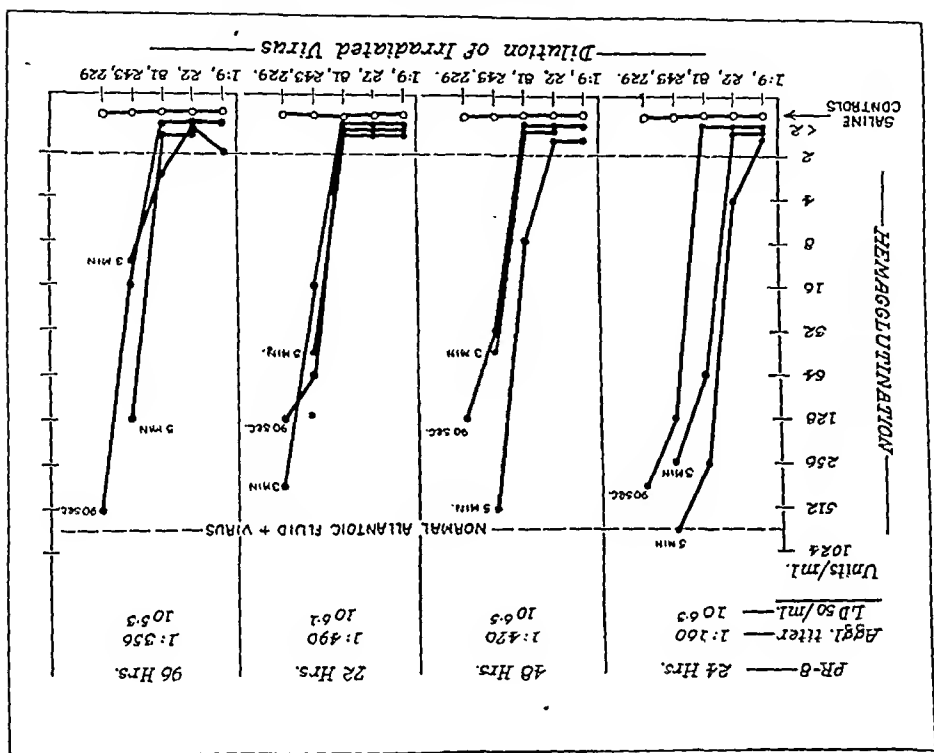


FIG. 1.—Relation between the incubation period of virus used for irradiation and the degree of interference.

*Length of Period of Protection.* In the experiments reported thus far, the interfering property was usually tested with active virus 14 to 18 hours after the primary inoculation of the irradiated material. In a few tests the active virus was given simultaneously with or immediately after the irradiated fluid, in others as late as 24 hours afterwards. The following experiment extends this range from 96 hours before to 24 hours after the inoculation of the test virus. Several hundred 8-day-old

embryos were received, and groups of them inoculated in the allantoic sac with undiluted or nine-fold diluted irradiated allantoic fluid infected with the PR-8 strain or with normal fluid, either on the same day or one of the three following days. On the 12th day of embryonic development one-half of the eggs in each group were injected with active virus and the other half with saline solution. In addition, a number of untreated 12-day-old embryos were inoculated with test virus to receive the interfering material 3 or 24 hours after the active agent. As shown in Table 3, all eggs inoculated with undiluted interfering fluid showed residual agglutination of red cells because of this infection. The titer in all these preparations did not differ markedly, in spite of the fact that the titer in the allantoic fluids harvested from other eggs immediately after the primary injection was about four-fold

TABLE 3.—LENGTH OF PERIOD OF INTERFERENCE

Age of embryo at 1st injection in days	Irradiated material injected	First injection			Time between 1st and 2nd injection in hours	Second injection		
		Hemagglutinin titer in allantoic fluid immediately after 1st injection	Hemagglutinin titer in allantoic fluid immediately after 1st injection	Hemagglutinin titer in allantoic fluid immediately after 1st injection		Hemagglutinin titer 48 hours following injection of	Saline	Virus
8	PR-8, undil. PR-8, 1:9 Normal fluid undil.	122	96	..	..	<2	12	384
9	PR-8, undil. PR-8, 1:9 Normal fluid undil.	90	72	..	..	<2	4	12
10	PR-8, undil. PR-8, 1:9 Normal fluid undil.	77	48	..	..	<2	8	16*
11	PR-8, undil. PR-8, 1:9 Normal fluid undil.	..	24	..	..	<2	32	16
12	PR-8, undil. PR-8, 1:9 Normal fluid undil.	60	..	..	..	<2	16	24
12	PR-8, undil. PR-8, 1:9 Normal fluid undil.	..	..	..	..	<2	32	16
13	PR-8, undil. PR-8, 1:9 Normal fluid undil.	30	..	..	..	<2	24	384
* Four out of 10 embryos infected.		..	..	..	..	<2	..	512
		..	..	..	..	<2	..	512
		..	..	..	..	<2	..	512

higher in the 8-day-old chick embryos than in the 13-day-old specimen. This drop in titer may be due in part to additional absorption of the agglutinins by newly developed cells of the allantoic sac, in part to the greater dilution of the hemagglutinins in the increasing volume of allantoic fluid during this stage of embryonic development, and finally, perhaps to a slight extent to destruction of hemagglutinins at 37° C., although test tube experiments did not reveal any loss of titer during incubation periods of similar length. As far as the interfering property was concerned, allantoic fluids from eggs receiving the irradiated virus as early as 96 hours before inoculation of the test virus did not show measurable amounts of hemagglutinin in the groups injected with nine-fold diluted interfering fluid, and no increase in titer in those prepared with the undiluted material. When the irradiated virus was injected 3 hours after the test virus, marked

reduction of hemagglutinin formation could still be obtained, while

after 24 hours no effect was noted.

*Removal of Free Irradiated Virus From the Allantoic Cavity.* In the

experiments thus far recorded, irradiated virus was injected into the allantoic cavity and frequently residual hemagglutinin could be demonstrated in the allantoic fluid of such eggs when harvested after various intervals of incubation. It was conceivable that the presence of this free irradiated virus in the allantoic fluid was responsible for the interference, and removal of it was considered essential. This was accomplished in a few experiments with slightly older embryos, *i. e.*, at the 12th day of incubation. Two holes were drilled just through the shell without injuring the shell membrane or underlying tissues. One of these was placed on one side from the embryo over the lower third of the allantoic sac, the other on the opposite side, 2 to 3 mm. under the air sac. Rubber disks of 4 to 5 mm. diameter and about 2 mm. thickness were pasted onto the shell over the holes with a heavy solution of collodion which was allowed to harden for several hours. The irradiated virus was injected through the lower hole. The egg was placed in an upright position and an 18-gauge needle with a short bevel was inserted through the same hole. This needle was connected by fine rubber tubing with a reservoir of buffered saline solution. In some instances the irradiated virus was injected into the lumen of the rubber tubing directly above the needle leading into the allantoic cavity. Through the opening under the air sac, an 18-gauge needle with one or two holes drilled into the side near the tip was inserted to serve as an outlet. The saline solution was then run through the allantoic cavity. By this technique amounts as high as 200 ml. have been flushed through in 15 to 20 minutes. However, in a number of cases much less was passed because of the clogging of the outlet needle with tissue. The effectiveness of this flushing was ascertained by determination of urates in the wash fluid, and it was found that the first three samples of 10 ml. each removed more than 93% of these substances, and after 60 ml. had been passed through the sac, only a fraction of 1% was left. This test actually may not reflect the true effectiveness of the flushing of the allantoic sac, since precipitates of urates are found on the membrane of embryos of this age and the removal of these may cause considerable difficulties.

After the allantoic sac had been flushed, the eggs were incubated again for 24 hours before the test virus was injected. The results of some of these tests are shown in Table 4. Only results from embryos flushed with 100 ml. of buffered saline solution or more are included. This amount was passed within 12 to 40 minutes. As seen in the table, eggs receiving only the injection of irradiated virus show a residual hemagglutinin titer in the allantoic fluid when harvested 72 hours later (eggs numbers 1 to 4). Eggs injected with irradiated virus, flushed thereafter with saline solution, and tested with virus 24 hours later did not contain measurable amounts of hemagglutinin within the allantoic sac when its contents were collected 48 hours after the inoculation of the test virus. This was the case when the flushing was begun

may have been left in the irradiated material which, however, may regard an analysis has to consider three possibilities: (a) active virus. Such an analysis has to consider three possibilities: (a) active virus. It is necessary, therefore, to review the experiments recorded in Evidence of this statement is included in the first paper of this series. up to 10,000 LD<sub>50</sub> per ml. before agglutination may be observed. high titer, *i. e.*, the infectivity for mice in an allantoic fluid may reach becomes positive only after the infectivity has already reached a fairly measure. In the technique used in this laboratory, the agglutinin test marked reduction in propagation of virus, but it is not an absolute to the development of agglutinins for chick red cells. Absence of with the propagation of the active agent have been discussed in regard of the development of agglutinins for chick red cells. Absence of

*Interference as Measured by Titration of Active Virus.* In the preceding pages various aspects of the interference of inactivated virus

* Irradiated virus injected into rubber tubing and flushing started at a rate of 10 ml. per min.		† Irradiated virus injected into rubber tubing and flushing started at a rate of 10 ml. per min.	
19-25 pool	Egg No.	19-25 pool	Egg No.
0	1	0	1
0	2	0	2
0	3	0	3
0	4	0	4
0	5	0	5
0	6	0	6
0	7	0	7
0	8	0	8
0	9	0	9
0	10	0	10
0	11	0	11
0	12	0	12
0	13	0	13
0	14	0	14
0	15	0	15
0	16	0	16
0	17	0	17
0	18	0	18

TABLE 4.—REMOVAL OF RESIDUAL IRRADIATED VIRUS FROM THE ALLANTOIC CAVITY AFTER INJECTION OF VIRUS

longer than 24 hours have not been tested yet. result of the procedures. Consequently, periods of protection of have been used, since there was too high a loss of younger embryos as a far only embryos of 12 days' incubation at the start of the experiment extended studies of 19 to 25). The mortality of the embryos undergoing this procedure is fairly high but it is hoped to improve the technique for more of eggs 19 to 25). The mortality of the embryos undergoing this procedure is fairly high but it is hoped to improve the technique for more showed the usual high titer in the hemagglutinin test (eggs numbers 9 to 14). Allantoic fluids of eggs not injected with irradiated virus but flushed with buffered saline solution and inoculated with active virus was started 30 to 60 minutes after the first injection (eggs numbers before or immediately following injection of the irradiated virus into HENLE, HENLE: VIRUSES OF INFLUENZA

have been prevented from adequately multiplying by an excess of inactive virus (interference); (b) the test virus injected into the allantoic cavity following the primary injection of irradiated virus, although prevented from infecting susceptible cells by interference, may survive in the allantoic fluid for a certain length of time at 37° C., and some of it may be present at the time of harvest 48 hours later; (c) in limiting dilutions of interfering fluid, finally, or after excessive irradiation of it, not all susceptible cells may be blocked by inactive virus, and the test virus may actually propagate. The last point does not require any further comment. The first two, however, were studied more closely. It will be shown that either development is possible and, in fact, both may coexist in one experiment.

TABLE 5.—DEMONSTRATION OF SURVIVING VIRUS IN IRRADIATED ALLANTOIC FLUID BY PROLONGED EXPOSURE TO ULTRA-VIOLET LIGHT OR BY DILUTION

Inoculum used for subculture (PR-8 virus)		Results obtained with allantoic fluids of subcultures	
Time of irradiation	Dilution of inoculum	Hemagglutinin titer in allantoic fluid	Infectivity for mice
3 min.	Und. 1:3 1:9 1:27 1:81	32 3 2 2 2	00000 2 = 0 D <sub>50</sub> = 00 3321 1 = 00 10 <sup>-1</sup> { Und.
5 min.	Und.	24	000000
10 min.	Und.	24	= 0000
20 min.	Und.	48	D <sub>50</sub> D <sub>7</sub> D <sub>1</sub> D <sub>5</sub> D <sub>3</sub> D <sub>9</sub>

*Survival of Active Virus in Irradiated Fluids.* Prolonged irradiation or dilution of the irradiated allantoic fluid may reduce the interfering property sufficiently to permit small quantities of surviving active virus to multiply and form measurable amounts of hemagglutinin (Exp. 5, Table 1, and paper 12, Table 6). Frequently the virus may not reach the level where agglutination of red cells becomes positive, but its presence may be ascertained by inoculation of either chick embryos or mice. Table 5 summarizes an experiment combining the two ways of demonstrating the presence of some residual active virus in irradiated fluid, namely, prolonged irradiation and dilution. As seen in the table, when infected allantoic fluid irradiated for 3 minutes was inoculated either undiluted or in dilution 1:3 by the allantoic route, some residual hemagglutinins were found on harvest, but not when higher dilutions were injected. When the allantoic fluids of the subcultures were inoculated intranasally into mice, those derived from the more concentrated inocula of irradiated virus did not cause apparent pulmonary lesions, while those from higher dilutions produced typical involvement. When the allantoic fluid was irradiated for 3, 5, 10, or 20 minutes and injected without dilution into eggs, all subcultures showed free hemagglutinins from this injection, but inoculation of mice with the fluids derived from virus irradiated for the shorter periods of time did not result in lung lesions, while those from virus treated for 20 minutes caused death with typical pathology. From this and similar experiments, it is apparent that at least 100 ID<sub>50</sub> of

active virus may be present in an irradiated fluid, which amount could escape detection if the fluid were injected undiluted only and if one depended on the hemagglutination test alone. In order to judge the degree of inactivation of virus by irradiation, therefore, extensive tests have to be performed.

*Survival of Virus Injected as Test Inoculum.* It was conceivable that some active virus from the test inoculation may survive long enough in the allantoic fluid to be demonstrable 48 hours later at the termination of an experiment. This assumption was verified in the following manner. Several hundred 10-day-old chick embryos were injected by the allantoic route with either irradiated PR-8 virus diluted 1:9, or with irradiated normal allantoic fluid in the same dilution. After further incubation of the eggs for 14 hours, some of the eggs in each group were tested either with active virus ( $10,000$  or  $1,000,000$   $ID_{50}$ ), or with saline solution. Of each group, 10 eggs were placed at  $-10^{\circ}C$ . immediately after the test inoculation, or after 2, 24, 48, and 72 hours of further incubation at  $37^{\circ}C$ . The allantoic fluids were collected aseptically after chilling of the eggs for at least 30 minutes and adequate samples of the pooled fluids from each group were stored in sealed ampules at  $-70^{\circ}C$ . until titration for virus activity in eggs could be performed.

The results of this experiment are summarized in Figure 2. In the left part of the figure the hemagglutinin titers have been plotted against the time of incubation while the right side shows the infectivity tests. The hemagglutination reactions gave the usual results. The irradiated virus had been diluted sufficiently to prevent complication of the test by residual hemagglutinins from the primary infection, but it was sufficiently concentrated to interfere with the formation of measurable amounts of hemagglutinin for at least 48 hours of incubation. After 72 hours the stronger test dose ( $0.5$  ml.  $10^{-2}$ ) gave slightly positive results in 8 out of 9 allantoic fluids. The allantoic fluids collected from eggs primarily injected with normal allantoic fluids gave the usual high titers in the agglutination test in 24 hours, and further increases were noted up to 48 or 72 hours of incubation.

When the allantoic fluids from eggs inoculated after the injection of interfering fluid with the smaller test dose ( $0.5$  ml.  $10^{-4}$ ) were titrated by the allantoic route, it could be shown that all contained active virus regardless of whether they were harvested shortly after the test inoculation or 72 hours later. The highest titers ( $ID_{50}$ /ml.) were found in the earliest harvests. With prolonged incubation, the titer decreased gradually until after 72 hours approximately 10% of the original amount could be demonstrated. The allantoic fluids from the groups of eggs inoculated with the larger dose of test virus seemed to follow the same general trend in the first few harvests, but the titer then remained more or less constant. The difference in active virus content between the groups tested with the two dilutions of virus were in the order to be expected, i. e., the allantoic fluid from eggs inoculated with the larger dose contained about 50 to 100 times more virus. Comparison of the fluids from eggs inoculated with the virus dilutions

after a primary injection of normal allantoic fluid showed that the findings reported in the preceding paper.<sup>2</sup> There was no significant difference in regard to the "0-hour-harvests" in the two corresponding series. However, in the next two hours a slight decrease in titer occurred in the two control series, signifying greater absorption of the virus as compared with the results in the interference-groups, which showed no such marked fall in active virus concentration. Whether or not this constitutes a significant difference between the experimental and control groups remains to be seen in further studies. Any

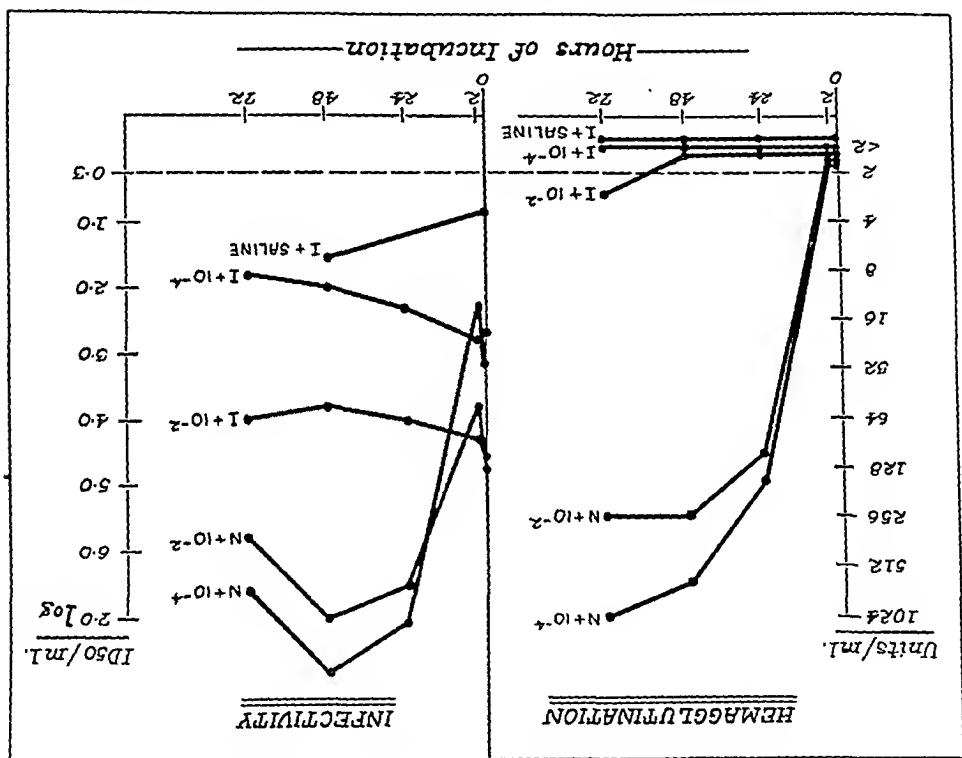


Fig. 2.—Demonstration of survival of active virus from test inoculation in the allantoic fluid for a period of 72 hours. N + 10<sup>-2</sup>, N + 10<sup>-1</sup>—normal allantoic fluid injected prior to test virus diluted 10<sup>-2</sup> or 10<sup>-1</sup>.

quantitative assay of absorption of the active virus is hampered by various factors. It is obvious that during the cooling down period of the eggs at -10° C. absorption of the virus may continue. However, this period was kept as closely similar as possible for the early harvests. Although the approximate amount of active virus injected was known, any calculations of how much should be recovered in the allantoic fluid of the injected egg are difficult in view of the fact that the amounts of allantoic fluid per egg during this stage may vary considerably, as also may the size of the allantoic sac and the number of susceptible cells. For this reason the apparent differences shown in Figure 2

should be considered only as preliminary observations until a more extended study has been made.

The experiment recorded shows definitely that virus from the test inoculation may survive in the allantoic sac for the period of time usually accorded to these interference experiments. Tests conducted *in vitro* were found to strengthen this observation. Concentrations of virus similar to those shown in Figure 2 were added to normal allantoic fluid in the approximate amount encountered in the eggs. The mixtures were kept in 3 ml. amounts in individual ampules at 37° C., and one each removed after the indicated time of incubation. The ampules were stored at -70° C. until the titration could be done. The results showed that with higher concentrations, about 1% of the virus may be active after 72 hours at 37° C. With smaller amounts of virus, the activity may be lost completely within 48 hours.

*Infection of the Allantoic Sac and the Embryo Following Injection of Irradiated Virus.* In the preceding experiments, interference was studied in regard to the appearance or prevention of the appearance of freshly propagated virus in the allantoic fluid. It was of importance to ascertain whether the irradiated virus injected prior to the fully active agent would prevent not only the accumulation of freshly propagated virus in the allantoic fluid, but whether it would protect, in addition, the embryonic tissues against infection. The following experiment shows that no active virus could be demonstrated in the allantoic sac nor in the embryo when sufficient amounts of irradiated virus were injected prior to the test virus. The experiment was similar to the one recorded in the preceding section of this paper (Fig. 2), but in addition to the allantoic fluids the allantoic sacs as well as the embryos were collected. These were repeatedly washed in large volumes of sterile buffered saline solution, ground with sterile powder and suspended in 5 volumes of buffered saline solution. After centrifugation at 2000 r.p.m. for 30 minutes, the supernatant fluids were saved and tested for virus activity together with the allantoic fluids collected from the same groups of eggs. Mice were used instead of chick embryos for the assay of infectivity. The 50%-infectivity end-point was calculated according to the method of Reed and Muench instead of the mortality end-point, since in the interference groups little or no mortality occurred. Only mice showing definite lesions were included in the calculations, while doubtful results ( $\pm$ ) were disregarded. The results of this experiment are summarized in Figure 3. As seen in the figure, the results of the agglutinative test (upper two charts) showed the usual findings. In the left half, where the interference groups are plotted, residual hemagglutinins from the primary injection of irradiated virus were demonstrable in the allantoic fluids from immediately after the injection until the termination of the experiment, during which period a slight decrease in titer occurred. The test virus injected 18 hours after the first treatment did not increase the hemagglutinin titer. Suspensions of membranes and embryos did not agglutinate red cells. The right half of the figure, which gives the control data, shows a rapid increase of hemagglutinins after the 12th hour of incubation in the allantoic



fluid and to a lesser degree in the suspension of allantoic membranes. The embryos, however, did not react in this test. The infectivity tests are plotted in the lower two charts of Figure 3. The irradiated virus did not produce demonstrable amounts of active virus in the allantoic fluid nor in the embryo or membranes (saline controls). Test injection of approximately 1000 ID<sub>50</sub> (for the chick embryo) into the eggs primarily treated with irradiated virus, showed again the survival of some of this test virus for 48 hours in the allantoic

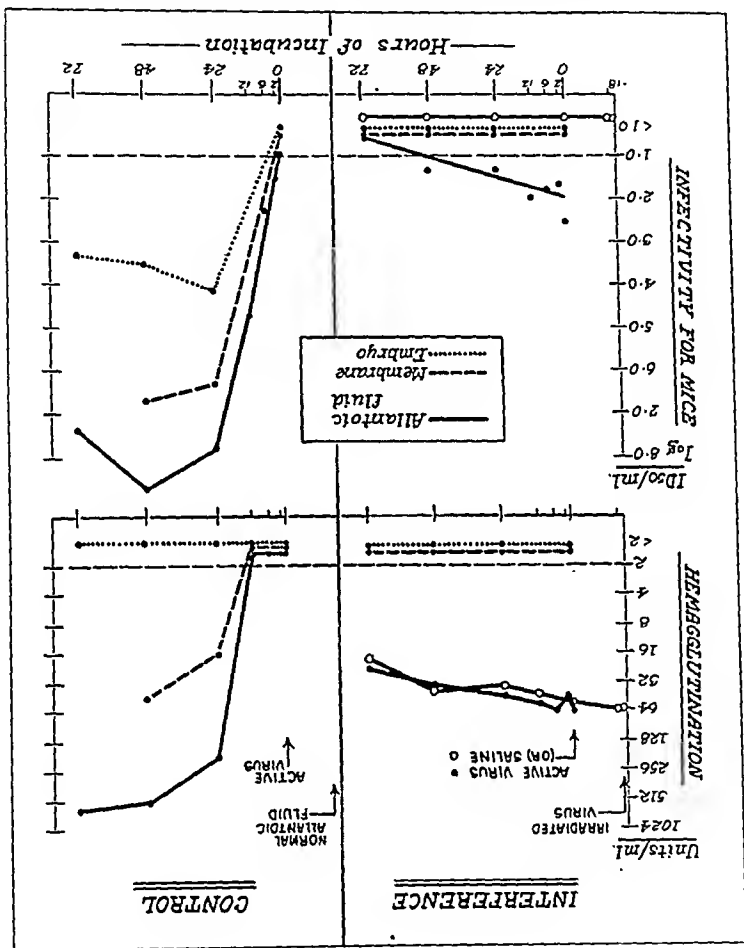


FIG. 3.—Prevention of infection of embryonic tissues by interference.

fluid. No active virus could be demonstrated in the membranes and embryos of these eggs. On the other hand, the controls, prepared by preliminary injection of irradiated normal allantoic fluid, revealed active virus in all three preparations. The highest infectivity titer was found in the allantoic fluid, followed by the membrane suspension and the embryos. This experiment showed, then, that the irradiated virus effectively blocked passage of the active agent into the embryonic tissues and protected them from infection.

**Discussion.** The interfering property of irradiated influenza virus with the propagation of the active agent was readily demonstrated in the allantoic sac of the chick embryo. This structure was particularly suited to such tests because of its even and relatively small total surface, in contrast to that of the complicated mammalian respiratory tract. Under optimal conditions, approximately 0.01 ml. of the irradiated allantoic fluids containing influenza virus prevented the formation of measurable hemagglutinins. This result depended somewhat on the technique of irradiation. Too intense or too long exposure destroyed the interfering property; too little irradiation left too much virus in the fully active state. The width of this range of interference, in turn, was dependent on the type of allantoic fluid used. Preparations harvested after incubationary periods of 24 or 48 hours (11- to 12-day-old embryos) lost their interfering properties more rapidly than fluids collected after longer periods of incubation, *i. e.*, after 72 to 96 hours (13- to 14-day-old embryos). This wider range of activity of the latter fluids rendered them more suitable for these studies.

Attempts to test for the degree of interference by titrating the active virus present in the allantoic fluids were complicated by several factors. The irradiated virus contained at times small amounts of active virus, and virus from the test inoculum may survive to some extent in the allantoic fluid of the eggs for the experimental period. As mentioned, irradiation of influenza virus under the conditions of these experiments did not always lead to complete loss of pathogenicity of the allantoic fluid. Although the injection of irradiated virus may not produce any symptoms in mice or formation of hemagglutinins in the allantoic cavity of the chick, second passage of either mouse lungs or allantoic fluid may show not infrequently that some active virus was still present in the original material. The experiments in the chick were on occasion quite paradoxical. The injection of less diluted irradiated virus may not yield any active virus on second passage, while injection of subcultures from the more dilute materials did. On the one hand, relatively large amounts of irradiated virus (1/100 ml.) were required to suppress formation of hemagglutinins. In contrast to this, 1/100,000,000 ml. of active allantoic fluid may still initiate propagation of virus, and it became obvious that only 1/100,000 to 1/1,000,000 of the virus had to survive irradiation in order to become demonstrable on dilution of the irradiated material. Smaller quantities of surviving virus (up to 100 ID<sub>50</sub>) could not be recognized under these conditions, since the inactive virus will interfere with its propagation. Such small amounts of the active agent, or any minute amount of freshly propagated virus did not retain activity at 37° C. for extended periods of incubation, as has been demonstrated by studying the survival of dilute inocula in the allantoic sac previously treated with irradiated virus. Small quantities of test virus may be inactive within 48 hours of incubation at 37° C., while with larger doses a certain percentage may survive for at least 72 hours.

Similar results were obtained on prolonged irradiation of the virus. The interfering property was gradually lost with increased time of

exposure, and was no longer demonstrable at a time when small quantities of active virus were left. It is obvious from these experiments that it is difficult to ascertain complete inactivation of a given preparation of influenza virus, and as long as only the undiluted irradiated 24 culture is tested, no reliable information is gained. These data will have to be considered in employing and evaluating vaccines inactivated by irradiation from the point of immunogenic activity and safety. The superiority of irradiated influenza vaccines as compared with formalized preparations has been demonstrated,<sup>4</sup> and may be due in part to surviving but not readily demonstrable active virus. As to the mechanism of this interference phenomenon, no definite explanation can be given at the moment. It is obvious that the inactivated virus is responsible for this effect. This statement is based on the following facts, which will be demonstrated more extensively in a subsequent paper. The interfering property is found only in infected allantoic fluids and not in normal preparations. It is non-dialyzable, is sedimentable in the high-speed centrifuge together with the active agent, and neutralization of irradiated influenza A fluids with specific anti-influenza A serum prevents the interference with the propagation of influenza B virus.

Hemagglutinins present in irradiated infected allantoic fluid are adsorbed to some extent by the cells of the allantoic sac. Attempts to ascertain the rate of adsorption have met with difficulties. Such determinations are dependent on the size of the allantoic sac and the amount of allantoic fluid present in the egg causing dilution of the inoculum; the strain from which the hemagglutinin was derived, and finally possible elution of the hemagglutinin from the cells.<sup>3</sup> In spite of these handicaps, a few results appear quite definite. Elution of the virus after adsorption could not be ascertained from consideration of the flushing experiments. Eggs inoculated with irradiated virus and flushed with buffered saline solution immediately afterwards, did not reveal any hemagglutinins in the contents of the allantoic sac 48 or 72 hours later. Unless elution occurred during the period of flushing, enough hemagglutinin could have been released to produce a positive test in these harvests. After adsorption of irradiated virus, no additional adsorption of virus takes place. Altogether, these statements are based on preliminary observations, and with improvement of the techniques employed, their validity has to be tested.

The flushing experiments have shown that the susceptible host cell may be changed very rapidly and the tissues may become refractory to infection with the influenza virus for at least 24 hours following contact with the irradiated agent. Since the test virus usually survives for more than 24 hours in the allantoic sac, the period of protection measured actually more than 48 hours. The change in susceptibility prevents the passage of the virus from the lumen of the allantoic sac into the membrane and embryonic tissues, but infection of the embryo by routes other than the allantoic does not inhibit the growth of the virus, as will be shown in a later publication.

**Summary.** Study of various aspects of interference between inactive influenza virus and the propagation of the active agent has given the following results:

1. Injection of approximately 0.01 to 0.005 ml. of irradiated virus (allantoic fluid) into the allantoic cavity will suppress formation of measurable amounts of hemagglutinin from a subsequent inoculation of active test virus.

2. The concentration of test virus has no influence on the results. They are similar regardless of whether 10 or 10 million  $ID_{50}$  are injected for testing.

3. No striking differences in interfering property were noted between irradiated allantoic fluids derived from virus cultures incubated for 24, 48, 72, or 96 hours. However, the interfering property in the earlier harvests (younger embryos) was more susceptible to destruction by ultra-violet light.

4. The interfering injection could be given as early as 96 hours before the test virus and up to 3 hours afterwards and produce extensive interference. When injected 24 hours after the active virus, no protection was noted.

5. Flushing of the allantoic sac with more than 100 ml. of saline solution following the injection of irradiated virus to remove free hemagglutinins, did not eliminate the interference. The protection of susceptible cells occurs very rapidly, since injection of the irradiated virus into the egg during flushing also prevented active virus from multiplying when injected 24 hours later.

6. Active virus frequently found in the allantoic fluid of the experimental eggs may be derived from 3 sources: (a) small amounts of virus may survive irradiation and enter with the first injection; (b) virus from the test inoculation may retain activity to some extent at 37° C. for the experimental period; (c) virus may actually propagate when limiting dilutions of irradiated virus were used for the primary injection. 7. Survival of active virus in irradiated preparations sometimes may be demonstrated only after dilution or prolonged irradiation of the allantoic fluid. Both steps decrease the interfering property and permit small quantities of surviving active virus to propagate.

8. The interfering injection prevents the test virus from entering and propagating in the embryonic tissues.

While these manuscripts were in press, an article by Ziegler, Lavin and Horsfall appeared,<sup>1</sup> which confirmed our observations previously reported.<sup>1</sup> These authors differentiated between partially inactivated and non-infective preparations of irradiated virus. This differentiation appears somewhat arbitrary in view of the fact that complete inactivation of influenza virus cannot be ascertained in the presence of the interfering agent. As a matter of fact, Ziegler and his co-workers come to the same conclusion when discussing their results. The findings reported by these workers are generally in good agreement with our results, with the exception that we failed to note differences in interfering properties of influenza A and B virus. The differences between the PR-8 and Lee strains recorded by Ziegler *et al.* might well be incidental findings, and other preparations of these strains might have given different results. One also has to consider that a certain amount of additional inactive virus may be introduced with larger doses of the active test virus (cp. Ref. 2), a phenomenon particularly noticeable with the Lee strain of influenza B

virus. Such small amounts of additional inactive virus could well be responsible for small apparent differences in interfering properties where no quantitative methods of hemagglutination are employed.

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## ONE YEAR OBSERVATIONS OF THE TREATMENT OF CANCER

## WITH AVIDIN (EGG WHITE)

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When our preliminary report<sup>10</sup> on avidin was presented about 1 year ago, it received some entirely undesired and regrettable newspaper publicity. As the authors stated at the time, the whole matter was still in the experimental stage and the procedure was not to be regarded as a cure. Unfortunately the lay public can rarely be made to understand that such experimentation must be carried on for a long period of time before any definite claims may properly be advanced, and thus it is that the simple announcement of any new procedure at once arouses unfounded hopes for a cure having been discovered.

Now that more than a year has elapsed since the experimental work with avidin was initiated, a year during which it was carefully and scientifically carried on, we feel warranted in making a further report so that both the profession and the laity may be informed of the results of our experimental investigation and an evaluation made of the proposed method of treatment.

In June 1942, following the reports of many workers<sup>2,3,8,11,19,20</sup> on the potent influence of biotin on cellular growth and the observations of others that egg white,<sup>1,6,13,14</sup> or more precisely, its constituent, avidin, possesses the ability to deprive the human system of biotin<sup>16</sup> and thus, as Laurence suggested,<sup>12</sup> possibly inhibit the influence of biotin on abnormal cell growth, it occurred to us to carry out some preliminary clinical trials as tests<sup>1,5,7,9</sup> of the hypothesis that malignant cells required biotin for their metabolic functions, and that egg white might seriously interfere with the vital function of malignant cells and thus bring about their destruction.

It was our thought to evaluate clinically this theory of control of carcinogenesis through diet, merely by substituting sufficient quantities of egg white in the diet of selected groups of patients, in place of a quantity of biotin-containing foods.

**Procedure.** As there was as yet no definite quantitative standard for measuring the amount of avidin required to neutralize biotin in the human system, the amount of egg white employed in these clinical experiments was necessarily chosen empirically, using Sydenstricker's<sup>18</sup> formula as a pattern.

For this experimental study,\* 10 cases were chosen, with definite specific malignant lesions, which could readily be visually and clinically identified, and the changes, if any, readily recognized. In 3 cases, avidin therapy alone was used; in the others, Roentgen ray therapy was employed in addition to the egg white treatment, in order to determine whether or not avidin increased the tumor radio responsiveness to Roentgen ray therapy.

The cases chosen were all proven by pathologic study to be malignant. They were: 2 cases of myelogenous leukemia, 1 of carcinoma of the stomach with belly wall metastases, 1 of melanosis of the leg with metastases, 1 of squamous cell carcinoma of the tongue, 1 of fibrosarcoma of the leg, 1 of Hodgkin's disease, 1 of cancer of the lung, 1 of metastatic neck cancer, and 1 of postoperative breast cancer with metastases.

The serious problem of feeding large amounts of egg white to patients was overcome by special care in the preparation of the diet, since in the beginning of this study a more concentrated form of avidin was not available. To carry out these experimental clinical observations, a number of beds were set aside exclusively for patients undergoing this treatment, and 2 trained graduate nurses were especially assigned in order to check properly every phase of the treatment. The amounts of egg white fed the patients were empirical and arbitrary, but as nearly as possible the amount suggested by Sydenhacker in his experiments for the production of biotin deficiency. Each patient ate 36 to 42 egg whites per day with a general diet as free as possible from foods rich in biotin, along with a daily supplement of accessory vitamins and minerals.

Viewing these cases as a whole, we noted that the general condition of all was markedly improved. Instead of the usual increasing cachexia commonly noted in such advanced malignant cases, a general well-being was evident, with improved appetite and willingness to be up and about. This was especially noted in 2 cases (tongue and stomach) which, previous to the treatment, were apathetic, morose and constantly complaining.

Despite the large amounts of protein ingested, no apparent disturbances in general metabolic processes were noted, uremia did not occur in any case and only in 1 case was there a temporary increase in the blood cholesterol. There was no significant change in the blood counts. (Cases 1 and 6), but although the high white cell count persisted, there was a noticeable relief of general subjective symptoms with avidin therapy. Although biotin deficiency effects have been reported as occurring in humans, none of these cases showed any definite symptoms of biotin deficiency.

With regard to the effect on tumor growth, in the cancer of the tongue (Case 7), we noted definite recession of the tongue growth. The tongue lesion, which filled the whole buccal cavity and prevented ingestion of all foods except moderate amounts of liquid, has receded to such an extent that the tongue is now freely movable and the patient able to eat semi-solid food. At present he can talk so as to be understood, whereas before treatment, mumbling was the best he could do. This patient was treated solely by avidin therapy. The small sub-maxillary nodes felt at the beginning of the treatment have in no way changed in their size or extent.

\* Aided by a special fund, obtained for us for this purpose through the efforts of Mr. William Laurence.

As already emphasized, while we felt that no definite conclusions could be drawn as to the therapeutic value *per se* of avidin, we nonetheless believed it worth while to continue carrying on our investigations.

One difficulty encountered has been that of determining the proper dosage of avidin required for controlling a specific malignant growth. At first we used as a daily dose of avidin, 36 fresh egg whites (about 126 gm.). Sydenstricker<sup>18</sup> in his experiments used 200 gm. of dried egg white powder, equivalent to 50 to 60 fresh egg whites. Whether or not a larger dose of egg white, avidin, is required to completely control malignant growth is still undetermined. We increased the amount of egg white to 42 per day and as far as can be observed there was little if any improvement in the results.

In March 1943, through the courtesy of Hoffmann-La Roche, Inc., we were able to secure a substantial amount of concentrated avidin and we have since then treated 1 case of cancer of the neck (Case 3), with a definitely proven, visible and palpable large metastatic squamous cell mass, solely with concentrated avidin. Each day this patient has been receiving 3 capsules of 666 mg. each of avidin, equivalent to a total of 2000 units per day. The diet was as near biotin-free as possible. As yet this patient has not exhibited any evidence of biotin deficiency. At first we noticed a decrease in the size and fixity of the tumor mass in the neck. Now after a period of 4 months and the ingestion of approximately 271,000 units of avidin concentrate, there is no evidence of tumor control or lessening of the neoplasia. The surface has broken down and involvement through the skin has taken place.

The 1 case of Hodgkin's disease (Case 4), a young woman 28 years of age, appears to have improved under egg white therapy alone. Although she still shows definite clinical evidence of the disease, she is about and working and in comparatively good condition, without complaints. While it is not, of course, entirely possible to attribute the improved condition of this woman solely to egg white therapy, nevertheless, we are not unwarranted in regarding egg white as having contributed towards her well-being and the inhibition of further advancement of malignant neoplasia.

The effect of avidin on radiation responsiveness is yet to be determined. However, the rapid response to Roentgen ray therapy in several cases suggests a definite influence of avidin. In 1 leukemia case, following avidin therapy, the radiation effect was definitely prolonged and the rise of white cell count controlled.

In the case of melanosarcoma of the leg and thigh with metastases irradiation after 3 months of egg white therapy caused complete disappearance of the clinical evidence of melanoma and has seemingly inhibited further extension or metastases.

In the lung cancer case, irradiation following prolonged egg white therapy appeared to produce rapid pulmonary changes and apparent resolution of part of the malignant lesion. However, this patient succumbed, probably to pulmonary hemorrhage (no autopsy being per-

(mitted) 10 months after treatment and undoubtedly later than if no treatment at all had been given, or at best the usual course of Roentgen ray therapy alone.

Details of all cases treated and under supervision follow.

**Case Studies.** Case 1. L.S., age 43, was admitted on August 24, 1942 complaining of a chronic cough of 5 to 6 years' duration which he attributed to his "sinus trouble." In 1938 blood-streaked sputum was noted, none since. In January 1942, following a cold, his cough became worse. Lost 12 pounds weight in 5 years. Because of continued cough he was admitted to the hospital. Examination on admission revealed questionable enlargement of the spleen, 1 cm.; Roentgen ray of the chest revealed moderate sized areas of infiltration at the extreme right apex and the peripheral portion of the right 1st interspace. In the axillary portion of the right 2nd interspace is an infiltrated area which stretches towards the mediastinal shadow by fibrous strands which give rise to some honeycombing effect. Left lung showed slight apical pleural thickening. Emphysema both bases. The trachea was markedly deviated to the right along with the remainder of the mediastinal shadow, but this was probably due for the most part to the eccentric position of the patient which was repeated on all films. Blood count showed W.B.C. 100,000. Diagnosis of pulmonary tuberculosis and myelogenous leukemia was made.

Patient, starting on eggs whites August 15, 1942, received 36 per day until December 4th. During the total of 112 days he received 4032 eggs whites. From December 5th until June 2, 1943 patient received 42 per day until 7434 eggs whites in 177 days were given. Thus this patient received a grand total of 11,466 eggs whites in 289 days. The patient also received Roentgen ray therapy over the anterior spleen on February 18th, and June 2nd, 300 r given at each sitting. Condition controlled.

Case 2. A.S., age 60, admitted August 18, 1942 complaining of cough productive of small amount of watery or mucoid sputum of 1 month duration. Three weeks before admission he began to sweat and developed pain in the right anterior chest. Weight loss of 25 pounds in 8 months. No wheeze, hoarseness or hemoptysis. Nocturia 6 to 8 times per night for past 6 years.

Examination at time of admission: Chest: decreased A.P. diameter, slight right apical lag. Lungs: dullness over upper lobe extending to 3rd rib anteriorly and 4th thoracic vertebra posteriorly. Absent breath sounds over this area, no rales. Rest of lungs clear. Nine sputum concentrations negative for tuberculosis. Roentgen examination of July 31, 1942 revealed: "Dense clouding in the medial two-thirds of the right upper lung field extending from the level of the 3rd rib anteriorly to the apex. Peripheral one-third is less densely clouded. Area is limited below by interlobar fissure which is convex upwards. Rest of lung fields are clear. Trachea is deviated somewhat to the right. There is some widening of the aortic shadow." Bronchoscopy on June 3, 1942 revealed: "Right upper lobe exceedingly difficult to identify. Eventually identified as a little crevice, its lumen completely occluded by thickened mucosa and also by compression from the outside. On the proximal lip of the right upper lobe there is some rough tissue." Pathologic examination of February 12, 1943 revealed squamous cell carcinoma of the bronchus.

Patient was started on egg whites August 10, 1942, receiving 36 per day until December 15, 1942. During 126 days he received 4536 egg whites. Starting December 29, 1942, patient received 42 per day until January 26, 1943. In a total of 35 days he received 1470 egg whites. Egg whites were again resumed on February 18, 1943, received 8 per day until June 2nd. He received 840 egg whites in 105 days. Thus since August 10, 1942 the patient received 6846 egg whites in 266 days.

From October 26 to November 24, 1942 Roentgen ray therapy was given to the anterior right mediastinum—3000 r, and to the posterior right upper chest—3000 r. From February 18 to April 2, 1943 Roentgen ray therapy was



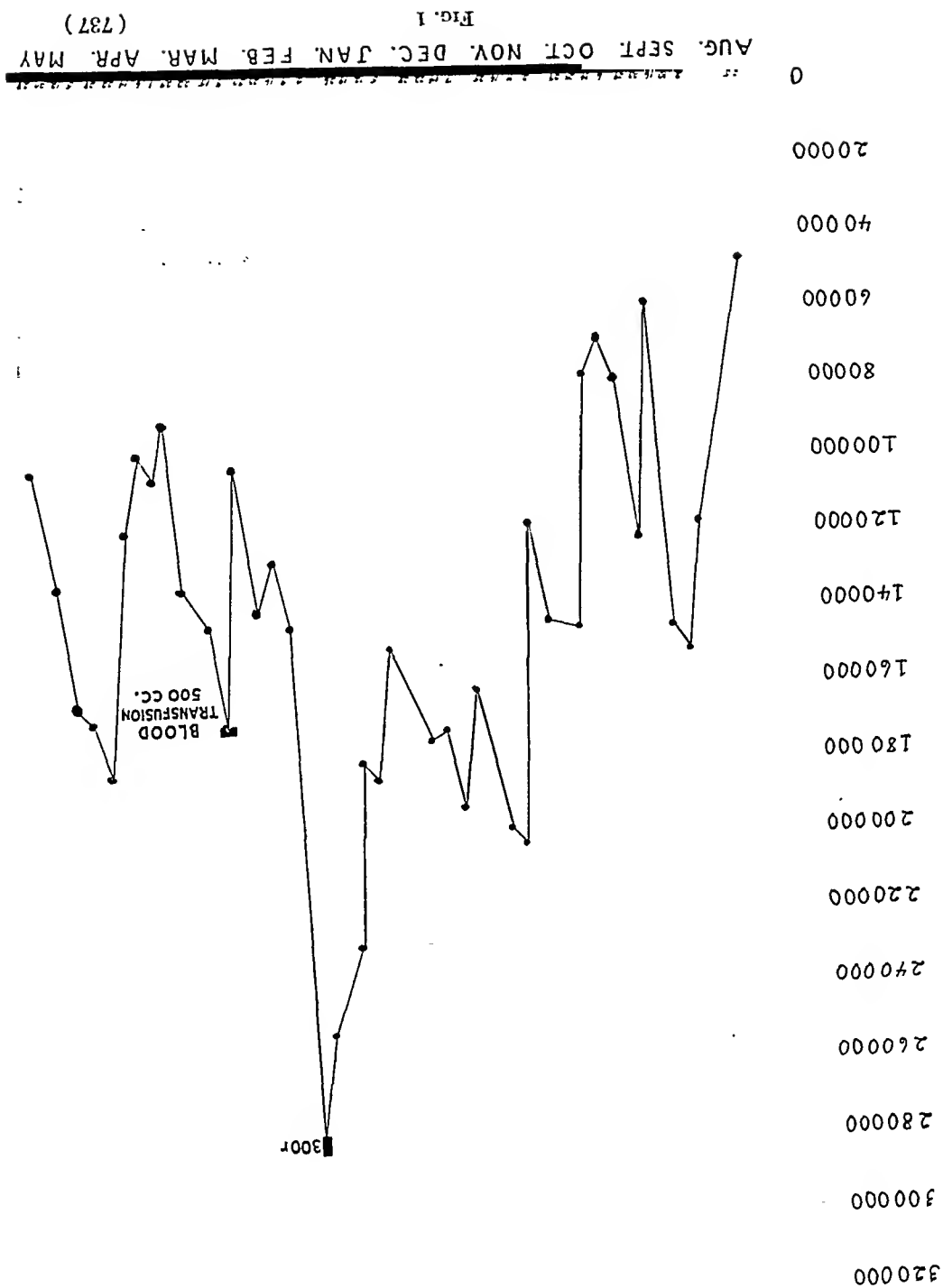
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~~Myelogenous Leukemia~~

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given to the anterior right mediastinum—3000 r, to the posterior right mediastinum—2100 r, and the right lower axilla—3000 r. On July 5, 1943 the patient expired from pulmonary hemorrhage. No autopsy was obtained.

Case 3. T.B., age 64, admitted March 6, 1943 complaining of a mass in the right neck of 3 months' duration. The patient is a fairly heavy user of alcohol and tobacco. He had gonorrhea 45 years ago, treated, lues denied. For 2 to 3 months before admission the patient states a mass has been growing on the right side of his neck below the ear and has been accompanied by occasional small hemoptyses. The growth has been progressive to the size of walnut and slightly tender. Patient has no chest pain, fatigue, dyspnea, dysphagia or change in voice.

Examination revealed the tongue to be smooth, posterior pharynx clear. In the neck there were no dilated veins or palpable nodes. On the right side just beyond the angle of the mandible and below the ear there was a solid, hard, irregular and non-tender mass the size of a walnut, not attached to the skin but movable below it. No signs of inflammation. Diagnosis of cancer of the neck, secondary, primary unknown was made.

Pathologic diagnosis of February 26, 1943 was squamous cell carcinoma, metastatic in neck, primary site unknown.

The patient was started on egg whites March 6, 1943, receiving 42 per day until April 1, 1943. During a total of 26 days he received 1092 egg whites. On April 1, 1943 the patient was started on avidin capsules, receiving 3 capsules per day until August 15, 1943. He received a total of 408 capsules of which 42 contained 660 units each; the remainder contained 666 units per capsule. The patient received 408 capsules in a 136-day period; 336 capsules contained 666 units each, equalling 223,776 units, the remainder, 42 capsules, contained 660 units each equalling 27,720 units. Thus from April 1 to August 15, 1943 the patient received a total of 271,476 units.

Case 4. G.C., age 28, was admitted November 9, 1942 complaining of enlarged gland and hoarseness of voice and cough of 3 months' duration. Patient stated that in August 1942 she began to cough and at the same time noticed swollen gland in the neck. Voice became intermittently hoarse. Examination at time of admission revealed 3 glands in the right supra-clavicular area varying in size from a pea to 1 cm. in diameter, discrete, firm, not fixed. No axillary adenopathy was present. Examination of the chest revealed mediastinal dullness percussed over widened area, particularly to right. No abnormal pulmonary findings were present. Abdomen revealed no tenderness or rigidity. Liver, spleen and kidneys were not palpable. Inguinal area revealed small, shotty node on left. Roentgen ray of the chest revealed several enlarged mediastinal nodes bilaterally, superior mediastinum.

Pathologic report (May 10, 1943): "Tissue consists of several lymphoid nodules the architecture of which is distorted by fibrous tissue. The lymphoid tissue shows hyperplasia of reticular cells and there are many large multinucleated cells of the Dorothy Reed type. There is an infiltration by polyps, plasma cells and eosinophils. There is marked perivascular fibrosis. Diagnosis: Hodgkin's disease of cervical node."

Patient was started on egg whites November 16, 1942 and received 36 per day until December 4, 1942, i. e., 648 egg whites in 18 days. Starting December 5, 1942, patient received 42 per day until January 30, 1943, 2394 egg whites in 51 days. Grand total of egg whites received in 75 days was 3042.

Case 5. S.S., age 44, admitted March 25, 1942 complaining of an unhealed ulcer of right leg for 4 months. The patient had a "stroke" in 1914 and 1915 with residual left-sided paralysis. He had a tumor of the right leg removed in November 1941. The patient stated that about 4 or 5 months before admission he noticed a painless swelling on the right foot which gradually increased in size and subsequently began to pain, especially when handled or accidentally injured. In November 1941 patient went to Harten Hospital where this lump was excised. Since this excision in November 1941 pain has increased and wound has not healed.

Examination of the patient revealed the cremasteric reflexes absent, Hoffman

positive right hand, Babinski negative. The left leg was thinner and weaker than the right. The dorsum of the right foot revealed an ulcer 4 x 8 cm. the contents of which moved with movement of the ankle. This was reddened, raised, swollen and ulcerated. Right inguinal nodes were palpable. Pathologic Examination (Harlem Hospital): Spindle cell sarcoma. Pathologic report from Bellevue Hospital (December 7, 1942): Spindle and giant cell sarcoma of the foot.

The patient was started on egg whites March 27, 1942, receiving 36 egg whites per day until October 24, 1942 (7560 egg whites in 210 days). Egg whites were resumed December 4, 1942, receiving 42 egg whites per day until March 23, 1943 (4904 egg whites in 112 days). On April 1, 1943 the patient started on egg whites again receiving 42 per day until May 1, 1943 (1260 egg whites in 30 days). Egg whites were again resumed on May 28, 1943 and he received 42 per day until June 22, 1943 with the exception of 6 days. In the total of 19 days he received 798 egg whites. Thus from March 27, 1942 until June 22, 1943 the patient received a total of 14,522 egg whites in 371 days. From June 2 to June 29, 1942 Roentgen ray therapy was given to the medial right leg—4230 r. From February 4 to March 24, 1943 Roentgen ray therapy was given to the anterior right foot—6000 r.

Because of combined contracture of the knee and distress amputation was performed on July 9, 1943. After amputation the patient has markedly improved and there is no evidence of recurrence or metastases. Case 6. V.H., age 68, was admitted August 15, 1938 complaining of bleeding from varicose veins of left foot. Examination revealed the spleen to be 10 cm. in mid-clavicular line. Two plus edema of ankles and varicose veins of both legs and on lateral side of left ankle a varix was sutured. Blood count showed typical myelogenous leukemia (W.B.C. 282,000).

Patient was started on egg whites September 3, 1942, receiving 36 egg whites per day until December 4, 1942 (3276 egg whites in 91 days). From December 5, 1942 until March 31, 1943 (116 days) she received 4872 egg whites. Thus she received a grand total of 8148 egg whites in 207 days.

The patient was also given Roentgen ray therapy over the anterior spleen on January 2, March 31 and May 28, 1943, receiving 300 r at each sitting. Her condition steadily improved.

Case 7. R.S., age 50, was admitted January 22, 1940 complaining of a rash on the right leg, spreading, of 6 months' duration. The patient stated that 6 months before admission she developed brown spots on the lower right leg which seemed to start from one brown pigmented area. She was admitted to the hospital and a diagnosis of Kaposis' disease was made. Biopsy was performed and reported "malignant melanoma." Examination revealed right inguinal adenopathy present, 2 cm. nodes, numerous oval, brownish, papular lesions scattered over the lower portion of the right posterior leg.

Patient was started on egg whites August 19, 1942 and received 36 egg whites per day until December 4, 1942 (3852 egg whites in 107 days). From December 4, 1942 to April 15, 1943 she received 42 egg whites per day (5502 egg whites in 131 days). Thus she received a total of 9354 egg whites in 238 days.

Pathologic report (October 15, 1942): Malignant melanoma of the thigh. From November 16 to 20, 1942 Roentgen ray therapy to right thigh, area 1, 500 r (superficial) and to right thigh, area 2, 500 r (superficial). From January 4 to 8, 1000 r; from January 18 to March 2, 1943, Roentgen ray therapy to single lesion on lateral knee 1500 r; to right heel 1500 r; to right thigh area 1, 1500 r; to right thigh area 2, 1500 r; to right thigh area 3, 1200 r. From March 3 to April 9, 1943 Roentgen ray therapy to right abdomen, 800 r; to right thigh areas 1 and 2, 1500 r; to right thigh area 3, 1500 r; to inner aspect of right knee 1500 r.

Patient was discharged home April 15, 1943.

On May 17, 1943, patient returned to clinic for observation. Examination showed that the local melanotic lesion in the right thigh had completely disappeared. No evidence of recurrence or extension.

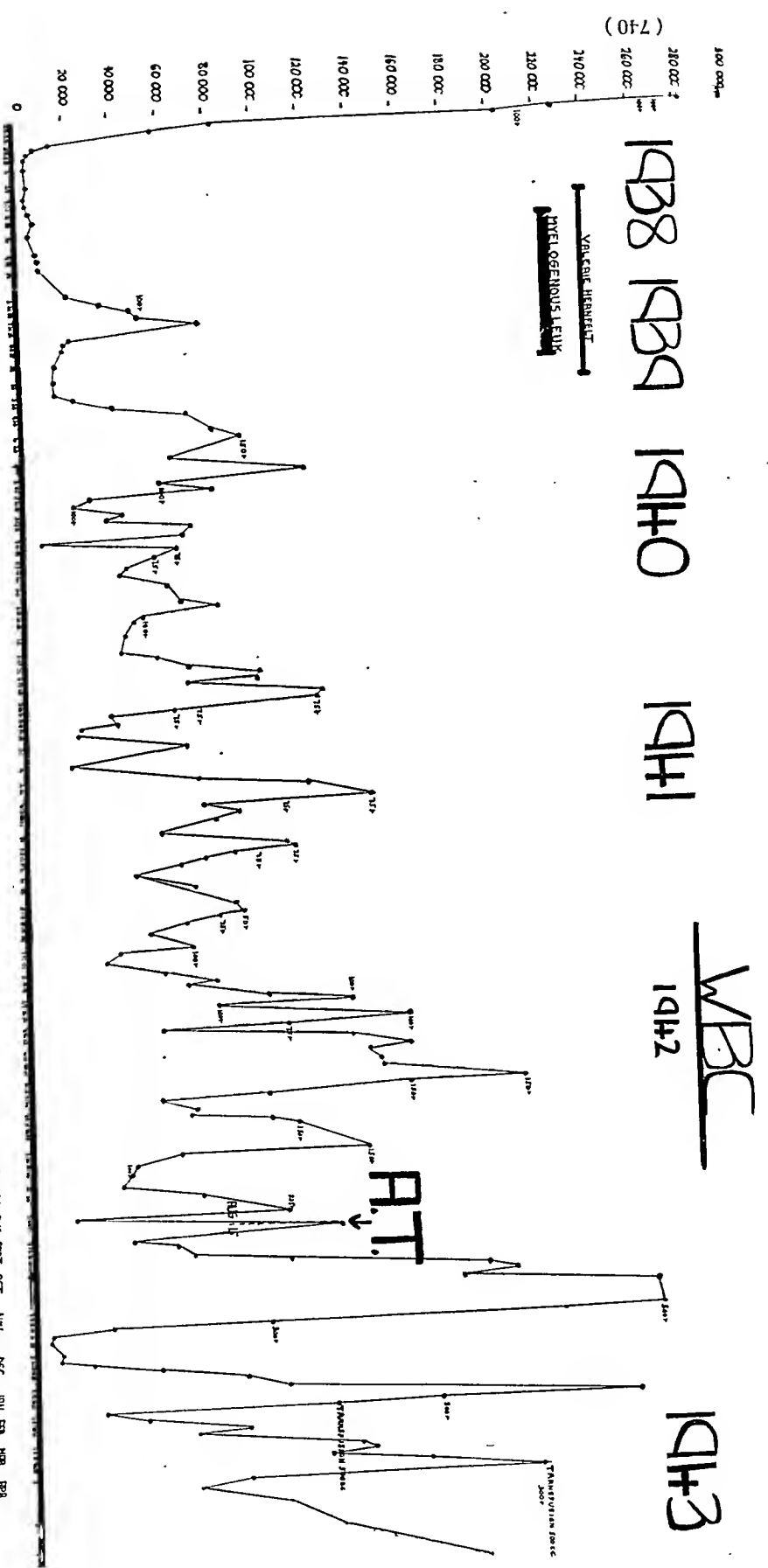


Fig. 2

Fig. 4.—A, Before treatment; B, after treatment.

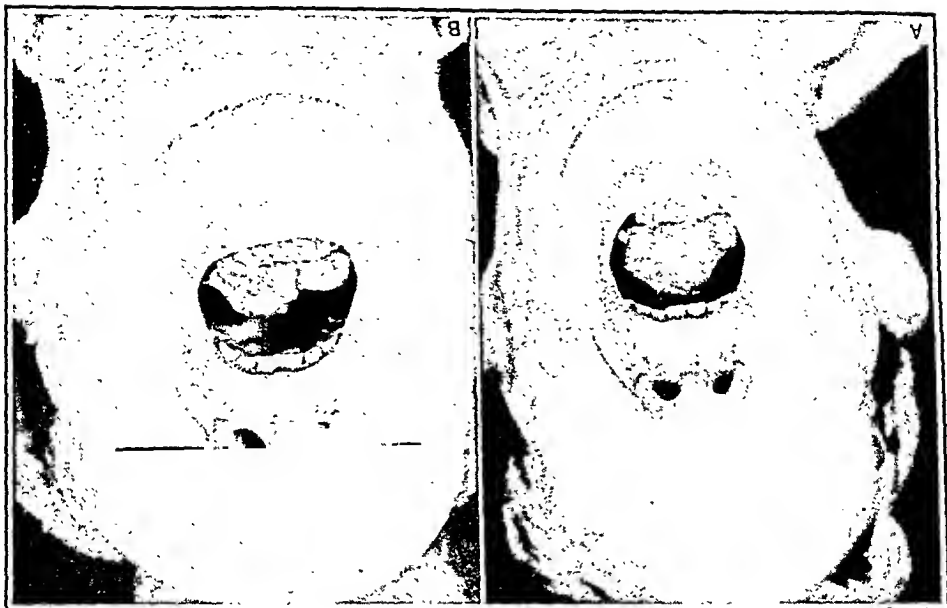
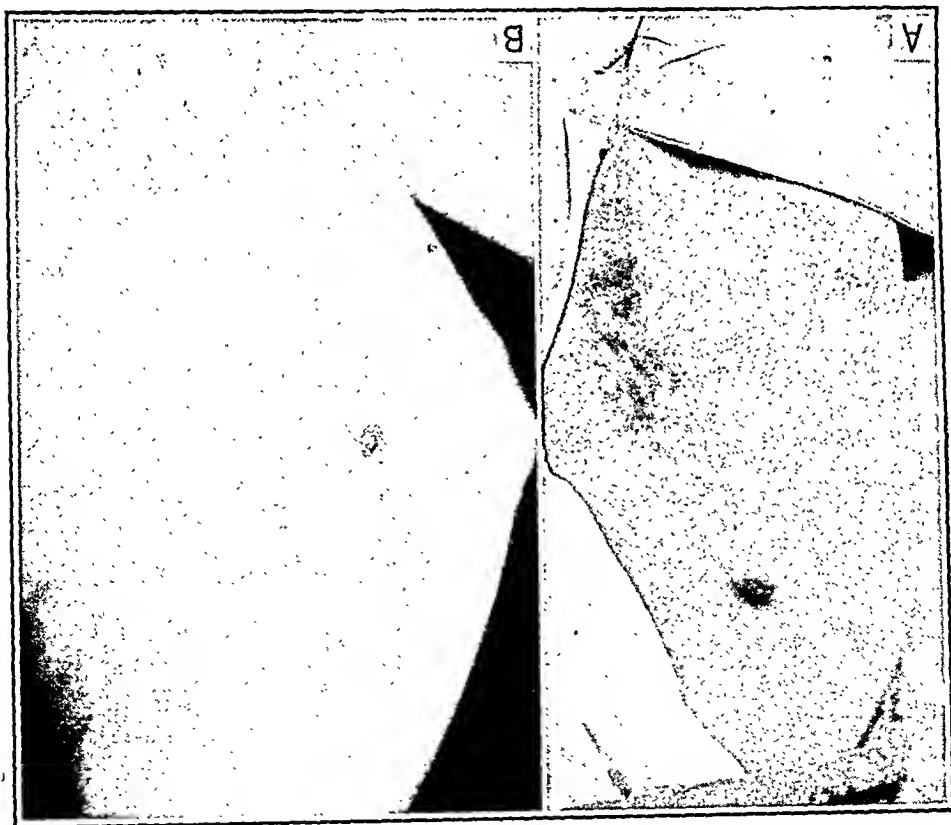


Fig. 3.—A, Before treatment; B, after treatment.



CASE 8. G.M., age 64, admitted August 17, 1942 complaining of difficulty in swallowing and talking of 4 months' duration and lesion on tongue of 6 months' duration. Six months previous to admission patient was aware of a small lesion on the left side of his tongue, posteriorly. Two months later patient began to have progressive difficulty in swallowing and talking and had pain in the left neck. Patient had noticed enlargement and recession of the mass in his tongue for a few weeks before admission. No smoking for past 25 years.

Local examination revealed the left half of the tongue occupied by a large fungating mass which extended from the lips to the posterior portion, crossing the midline. There was an ulcerated trench adjacent to the lower row of teeth, at the side of the tongue. Tongue was fixed and could not be extruded beyond the lower incisors. No acute infection was present. There was a solitary hard nodule in the region of the right submaxillary gland about  $2 \times 2$  cm. A firm left tonsillar gland about  $2 \times 1$  cm. was present with marked tenderness in the neck just below it. No further adenopathy was present. Patient had offending teeth removed on left side.

Pathologic report (August 19, 1942): Squamous cell carcinoma of tongue. Patient started on egg whites August 15, 1942 and received 36 egg whites per day until December 4, 1942 (total of 111 days—3996 egg whites). From December 5, 1942 to March 28, 1943, he received 42 egg whites per day (4788 egg whites in 114 days). April 5, 1943, patient resumed egg whites again, receiving 42 egg whites per day until July 8, 1943 (3790 egg whites in 90 days). Thus since August 15, 1942 the patient received a grand total of 12,564 egg whites in 315 days. On July 8, 1943, the lesion was more than 50% smaller and the patient could talk clearly and swallow freely.

**Discussion and Conclusions.** We can state that as yet no definite cure of cancer has been effected through the use of egg white therapy as employed by us in a selected group of cases. As the diet given contained substantial quantities of milk which may have been a source of readily absorbable biotin, this may have been one reason for the unfavorable results obtained. But while no definite conclusions can as yet be predicted on our results, based on a large number of cases over a considerable period of time, we believe that avidin must be given in larger doses than those we employed, either in the raw egg white state or in avidin concentrate with the diet quite free of biotin. Whether the neutralization by avidin of the cell-growth stimulating biotin accounts for the effects already noted cannot be stated definitely, but the fact that the customary progressive extension of the malignancy in several cases is absent inevitably leads to the conclusion that avidin therapy may have played a pronounced rôle in such cases. Notwithstanding that the results obtained do not prove substantial value of egg white treatment of neoplastic diseases, it would not be proper to draw therefrom any definite conclusions evaluating this method of treatment. This is because we were unable to produce biotin deficiency in our patients even by the employment of pure avidin in the amount of approximately 2000 units per day. It remains now to improve the method of treatment with vitamins so as to balance the diet to such a degree that by addition of avidin a biotin deficiency would be created. The proper dosage for avidin still waits to be determined.

Dr. Milton Zurrow who ably assisted in the early stages of the work concluded his internship in our service some months ago, prior to the conclusion of the study, and his place was taken by Dr. John Moseley. The author's thanks are extended to both.

For the financial aid which made this research possible we are indebted to Mr. William Laurence of *The New York Times*, and to the Carter Nicholas and Burden Funds. Further support from these sources enables us to continue the experiment and we expect to have further results to report at a future date.

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## CONCENTRATION OF RED BLOOD CORPUSCLES CONTAINING LABELED PHOSPHORUS COMPOUNDS IN THE ARTERIAL BLOOD AFTER THE INTRAVENOUS INJECTION\*

### PRELIMINARY REPORT

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To the Memory of Soma Weiss

Since the time of Stephen Hales, but few until very recently have studied the velocity of the blood flow in man. The most notable studies were made by H. Blumgart and Soma Weiss,<sup>1</sup> 1927-1928, who introduced a new method of measuring the velocity of the blood flow. They injected radium C intravenously and, with the aid of the Geiger counting-chamber, determined the circulation time in different parts

\* We wish to express our appreciation to Professor Hevesy for his constant advice and encouragement and especially Professor Rundström, who has generously placed at our disposal his laboratory. We also wish to express our hearty thanks to Prof. M. Stegbahn for the gift of radioactive phosphate.

of the circulation. Thanks to many published contributions they founded our knowledge of the velocity of the blood flow in normal and pathologic conditions, especially in compensated and decompensated heart cases. Their results have been confirmed by Tarr, Oppenheimer and Sager,<sup>9</sup> Winternitz, Deutsch and Brill,<sup>10</sup> Nylin,<sup>7</sup> Nylin and Malmström,<sup>8</sup> and others.

With the aid of other test substances, especially decholin, the fundamental investigations of Blumgart and Weiss have produced results of practical importance. We now know that a prolonged circulation time in heart cases means a slow circulation and is a criterion of decompensation. But Nylin<sup>7</sup> and Nylin and Malmström<sup>8</sup> have shown that it is not only the slow circulation in decompensation that explains the prolonged circulation time, but that there is another factor that must be taken into consideration. Nylin<sup>7</sup> has shown that the amount of the residual blood in the heart plays a dominating rôle in prolonging circulation time. There are many heart cases without any signs of decompensation, where all congestive phenomena are absent, with normal venous pressure and no congestion of the lungs, but with dilatation of the heart and a pathologically great heart volume, in which the circulation time is markedly prolonged. At the same time the duration of the taste sensation of decholin is greatly prolonged in those cases. Nylin<sup>7</sup> has postulated, and found in fact, that the prolonged circulation time and the long duration of the taste sensation in compensated heart cases are closely correlated with the increase of residual blood, as would be supposed from the pathologically increased heart volume. The determination of the velocity of blood by the aid of decholin is a subjective method and gives no real quantitative information. For the purpose of reaching a better understanding of this problem, Nylin and Hevesy, in Copenhagen, have introduced a new method for determining the red blood corpuscle contents. Thanks to Hevesy's generous permission, one of us (M. M.) has learned in his laboratory the technique of labeling the corpuscles and of measuring the activity with the aid of the Geiger counter. In Stockholm, Malm has introduced the method in Wenner-Gren's Institute, and Nylin has applied it in clinical research.

**Experimental Procedure.** Hitherto in experiments performed on human objects the indicator is injected directly in the blood. By analysis of the collected blood samples a measure of the amount of indicator in the plasma is obtained, because a permeation of indicator in the blood corpuscles during the short times required by the experiments could be neglected. After a strong rising at first of the concentration of the indicator in the first blood samples, the concentration is rapidly falling in the following samples not only due to the rapid mixing of the indicator with the blood but also due to the rapid resorption of the indicator by the tissues in the body. This fact is also pointed out by Gerlach *et al.*,<sup>2</sup> who has determined the time for circulation of the blood in man with the aid of Th X. In the mentioned work this also appears from a graphic registration of the lapse of time for mixing of the indicator with the blood. According to a method to determine the content of the red blood corpuscles worked out by Hevesy and Zerah<sup>1</sup> this source of error is eliminated. This modification of the earlier described procedure<sup>3</sup> was worked out just in view of a possible clinical application of the method.



By the method mentioned above, both plasma and blood corpuscles are labeled, but only the amount of indicator of the latter is determined after the injection. This method has been followed in the present work.

Five ml. of blood are removed from the patient, placed in a small flask containing a negligible weight of sodium phosphate labeled with radioactive phosphate. The flask is shaken in a thermostat for 2 hours at 37° C. After this time the distribution coefficient of labeled ions between corpuscles and plasma of equal weight has reached a value of about 0.9.<sup>4</sup>

Three ml. of the blood containing the labeled corpuscles are then reintroduced into a vein in the arm of the patient. Before the injection, a cannula, to which was attached a tap with a rubber tube, was introduced into an artery of the other arm, which made it possible to collect the blood samples only a few seconds after the injection. The blood is collected in small glass tubes—about 2 or 3 ml. in each.

The samples were then centrifuged, the plasma is removed and about 0.5 gm. blood corpuscles are weighed in. The blood corpuscles are ashed with sulfuric and nitrogen acid, a known amount of inactive sodium phosphate is added and the phosphate is precipitated as ammonium magnesium phosphate.<sup>5</sup> The obtained precipitates are measured with the aid of a Geiger counter.

The strength of the radioactivity in the different samples is worked out per gram and is expressed in % of the value of the strongest sample.

Possible errors such as adhesion of active plasma to the corpuscles and how properly the label of the corpuscles is conserved are discussed by Hevesy and Zerahn.<sup>6</sup>

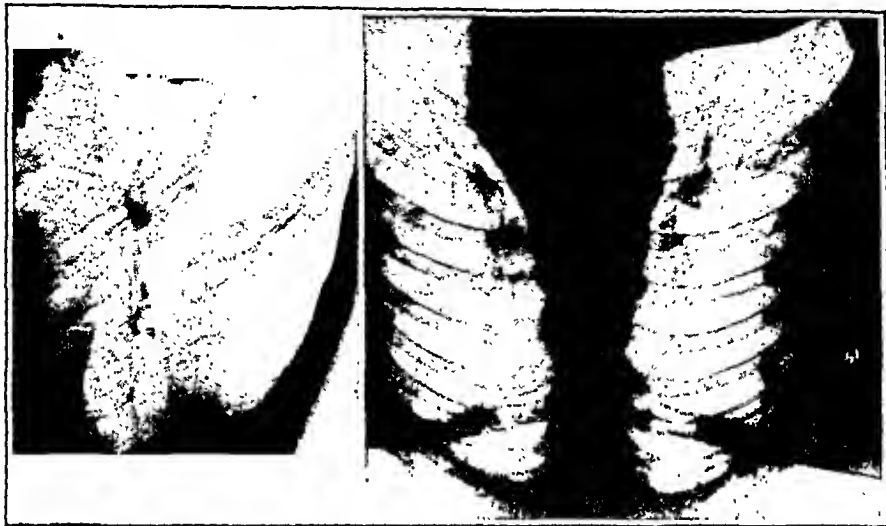


Fig. 1.—Heart volume in a healthy person in a standing position. Volume 620 ml., i. e., 320 ml./m<sup>2</sup> of body surface. (Case 1.)

**Results.** Since November, 1942 we have applied the method in some clinical cases in order to find out whether the prolonged circulation time and the prolonged duration of the taste sensation, determined by the aid of decholin, in cases with heart dilatation, were correlated with a prolonged relative activity of the arterial blood after injection of red corpuscles containing labeled phosphorus compounds. Examples of these results follow.

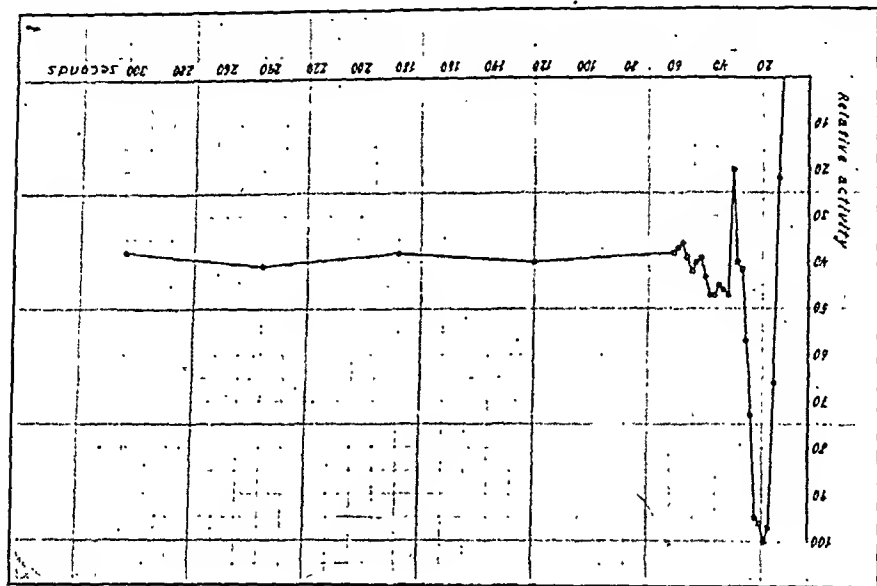
**Case 1.** Healthy man, 27 years of age. Heart examination normal. Blood pressure 120/80. Venous pressure 7.5 cm. Velocity of circulation,

determined with decholin: first taste sensation after 15 seconds, disappearance of this sensation after 34 seconds, after intravenous injection of decholin. Roentgen ray of the heart: Heart configuration normal. Heart volume 620 ml., i. e., 320 ml./m<sup>2</sup> of body surface (Fig. 1).

TABLE 1.—VALUES OBTAINED IN CASE 1

Time after injection, sec.	Relative activity	Time after injection, sec.	Relative activity	Time after injection, sec.	Relative activity	Time after injection, sec.	Relative activity
5	0	33	19.7	120	40	1 day	47
7	0	35	47	180	37.5	3 days	13.5
9	0	37	46	240	41.5		
11	0	39	45	300	37.5		
13	21.6	41	47	600	33		
15	66	43	47	900	34		
17	86.5	45	43	1800	40		
19	100	47	38.5				
21	96	49	40				
23	95	51	41.5				
25	73	53	38.5				
27	57	55	35.6				
29	42	57	36.5				
31	.	60	37.5				

CHART 1.—Relative activity of the blood in the brachial artery as a function of time in a healthy person. (Case 3.)



Intravenous injection of about 10  $\mu$  C. in the right antecubital vein was made early in the morning with the subject in a horizontal position. The subject had not taken any food since the previous evening and had not been out of bed in the morning before the test. During the injection in the right arm and during the puncture of the left brachial artery, both arms were held in a quite horizontal position. The puncture of the artery was made, before the intravenous injection in the right arm, with a syringe cannula of the size we use for venous pressure determinations. A tap was attached to the needle, and to this a rubber tube. Cannula and rubber tube had been moistened with heparine to prevent blood clotting. Immediately after the labeled corpuscles had been injected very quickly (in about a second), the tap of the cannula in the artery was opened, and small glass tubes moistened with heparine and

placed in a special stand, were filled up to about 2 ml. each with blood from the artery. It took about 2 seconds for each tube. The blood in the tubes was then analyzed for its activity. The values of the relative activity are inserted in Table 1 and also in Chart 1. From this diagram it can be seen that the labeled corpuscles make their first appearance in the arterial blood about 13 seconds after the intravenous injection, and that the relative activity increases to its highest point 19 seconds after the injection and then it rather quickly decreases to a minimum 33 seconds after the injection. This means that there is a strong correlation between the measurements of the velocity made with decolin, when the first taste sensation occurred 15 seconds after the injection and disappeared after 34 seconds, and the relative activity of the arterial blood. In the diagram there is a new peak in the curve after 35 to 43 seconds as a result of recirculation. After that time the curve rather quickly decreases to a constant level, which persists for more than 300 seconds.



Fig. 2.—Heart volume in a case of mitral stenosis with extreme bulging of the pulmonary artery. Heart volume 2,490 ml., i. e., 1,600 ml./m<sup>2</sup> of body surface. (Case 2.)

Case 2. Woman 51 years old. Diagnosis: Mitral stenosis without congestion with severe heart dilatation. Blood pressure 170/110. No symptoms of congestion. Venous pressure 8 cm. Velocity of the blood flow determined with decolin: first taste sensation after 20 seconds; disappearance of the bitter taste after 90 seconds. Roentgen ray of the heart shows a generalized cardiac enlargement with pronounced bulging of the pulmonary artery. Heart volume 2,490 ml., i. e., 1,600 ml./m<sup>2</sup> of body surface. (Fig. 2.)

The results of the blood velocity studies with labeled corpuscles are seen in Table 2 and Chart 2. From Chart 2 it is seen that the relative activity of the arterial blood in the left brachial artery increases slowly to a maximum 25 seconds after the injection of the labeled red corpuscles in the right antecubital vein, and that then during a much longer time than in the normal case, there is a rather high level of the relative activity of the arterial blood—more than 120 seconds—and that during a time of 30 to more than 60 seconds there are small, irregular peaks in the curve. After 180 seconds the curve reaches a more constant level, which is maintained for more than 300 seconds after the injection.

Comment. From these preliminary investigations it is seen that, in a normal person with a normal heart volume, the velocity of the blood flow—determined with the aid of red corpuscles containing labeled phosphorus compounds—is strongly correlated to the circulation time

and the duration of the taste sensation after the injection of decholin. To some degree this correlation also holds good in the case of heart dilatation, and Chart 2 clearly shows that it takes a remarkably long time for the activity in the arterial blood to reach a constant level after the injection. In the case of heart dilatation without signs of congestion this slow decrease of the first part of the curve is probably

TABLE 2.—VALUES OBTAINED IN CASE 2

Time after injection, sec.	Relative activity	Time after injection, sec.	Relative activity	Time after injection, sec.	Relative activity	Time after injection, sec.	Relative activity
5	.	31	76	57	59	79	76
7	.	33	76	61	73.5	76	73.5
9	.	35	85	63	72.5	78	75
11	.	37	83	65	72.5	79	75
13	.	39	83	67	72.5	80	75
15	.	41	82	69	72.5	82	75
17	.	43	92	71	72.5	84	75
19	.	45	88	73	72.5	86	75
21	.	47	78	75	72.5	88	75
23	.	49	70	77	72.5	90	75
25	100	51	82	79	72.5	92	75
27	.	53	74	81	72.5	94	75
29	.	55	72.5	83	72.5	96	75

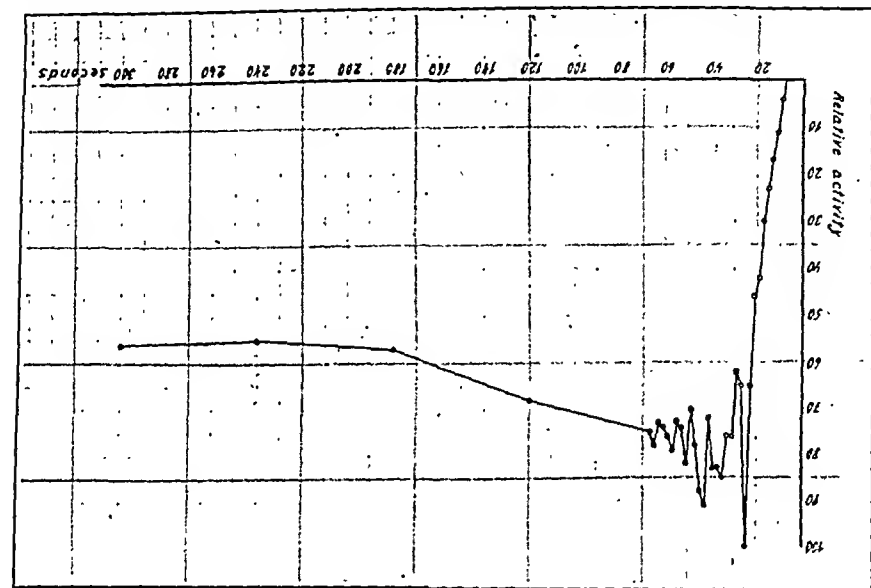


CHART 2.—Relative activity of the blood in the brachial artery as a function of time in a case of heart dilatation without congestion. (Case 2.)

to be explained by a great amount of residual blood in the heart and probably by the mixture of the injected labeled corpuscles with the residual blood not being so homogeneous and sudden as in a normal case. The irregular peaks of the curve in the case of heart dilatation also speak in favor of the assumption that, with a high amount of residual blood, the mixing of the blood in the heart takes a much longer time than in a normal subject. After this preliminary report,

it seems worth while to make further studies—which are actually proceeding in my laboratory—to gain more information about the amount of the residual blood in different heart cases. There seems to be some evidence for the assumption proposed by Nylin that the velocity of the blood flow and the prolonged circulation time are determined not only by the degree of decompensation, but probably also to a great extent by the amount of the residual blood in the heart. Summary. 1. Hevesy has introduced a method for the determination of the erythron of the rabbit in a simplified form. 2. We have applied this method to man, *i. e.*, to healthy persons as well as to patients with heart dilatation. 3. There seems to be a correlation between the amount of the residual blood in the heart and the relative activity in the arterial blood of labeled red corpuscles determined at certain fixed points after intravenous injection of these corpuscles.

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## THE EVALUATION OF ALCOHOL LUMBAR PARAVERTEBRAL BLOCK IN PERIPHERAL VASCULAR DISEASE

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The use of alcohol to produce block of the sympathetic ganglia was first introduced in this country by Swetlow<sup>5</sup> in 1923. Since then, various workers<sup>4,6</sup> in the field of peripheral vascular diseases have used this procedure to induce vasodilatation in the lower extremities and obtain relief from ischemic symptoms and signs. No one has as yet determined objectively how long this vasodilatation exists. White<sup>7</sup> mentions that the effects of alcohol lumbar paravertebral block rarely lasts longer than 6 months. In the past, particularly in the cases of angina pectoris, the evaluation of the effect of such injections has been made according to the patient's subjective feeling and, as in other evaluations, the efficacy of a therapeutic procedure. It is well known that symptoms of vascular

disease may be relieved spontaneously, and so we thought it would be important for us to know by an objective method exactly how long one may expect the vasodilatation in the lower extremities to last after the injection of the lumbar vertebral ganglia with alcohol.

White<sup>9</sup> has shown both experimentally and in humans that, as a result of injection of 5 cc. of alcohol into tissues, an area of necrosis 1 cm. in diameter will be produced. If this necrotic area should involve the exact spot of the sympathetic ganglia, then it is conceivable that the effect of the injection might last forever.

We therefore tried to determine objectively: (1) exactly how long one may expect vasodilatation effects to last after alcohol paravertebral block in the lumbar region; (2) whether neuritis is produced by such a procedure, and if so, how often, how severe, and how long such neuritis might last; (3) whether the amount of alcohol used was a factor in producing vasodilatation or neuritis; (4) whether the use of novocaine in sweet almond oil would reduce the incidence of neuritis;,\* (5) incidentally, we tried to determine if claudication time would be altered by such therapy.

**Procedure.** *Material.* The patients studied were those who applied to the vascular clinic for relief of symptoms, and comprised a total of 16 patients. The types of cases were as follows: 1 phlebitis, 1 scleroderma, 1 thrombo-angitis obliterans and 13 arteriosclerosis obliterans. These cases were chosen because it was determined previously, by peripheral nerve block with novocaine, that the extremity to be treated could vasodilate up completely.<sup>1</sup> These patients, therefore, served as excellent controls, for if our technique in doing the paravertebral block was correct, these patients should dilate up to 31° C.

Using the technique described by previous workers,<sup>1,6</sup> we injected the regions of the first, second and third lumbar vertebral ganglia. We divided the series into 3 groups.

Group I. Six cases: We used 2% novocaine followed by 5% novocaine in sweet almond oil, and this in turn by 1 cc. of 100% alcohol.

Group II. Four cases: 2% novocaine followed by 1 cc. of 100% alcohol.

Group III. Six cases: 2% novocaine followed by 3 cc. of 100% alcohol.

The blocks were performed at the clinic, the patients receiving no sedation. The patient was in the prone position during the block, with a pillow under the abdomen. The lower extremities were exposed from the thigh to the toes. The patient was kept in the prone position for about  $\frac{1}{2}$  hour after the block and then turned over on the back. Skin surface temperature readings were taken 1 hour before injection to allow the skin temperature to stabilize. Readings were taken at intervals for a period of 2 hours after the block, at the end of which time the patient was allowed to leave the clinic.

Skin surface temperature readings were taken bilaterally: (1) at the base of the nail, on the dorsum of the big toe; (2) the mid-sole region of the foot; (3) the external malleolus; (4) the head of the fibula.

Follow-up skin surface temperature readings were done at various intervals; at first 3 days, and subsequently at longer intervals. A temperature difference of 2° C. in corresponding areas of extremities was considered significant. When there was no further significant difference in temperature in corresponding areas, the vasodilating effect of the injection was taken to have stopped, and the follow-up was ended.

\* The use of 2% novocaine in sweet almond oil was suggested by Dr. Sidney Gross, to whom we are indebted for the demonstration of the technique of lumbar paravertebral block.

† The Taylor Dermatoherm was used throughout our experiments.

When taking the follow-up readings, the patient was seated with the legs in the horizontal position, and the lower extremities were exposed to room temperature for 1 hour, at the end of which time readings were taken. We attempted to keep the room temperature constant at any one sitting, but the room temperature at different times was as high as  $26.6^{\circ}\text{C}$ ., and as low as  $18.6^{\circ}\text{C}$ .

**Rationale of the Evaluation of the Alcohol Paravertebral Block.** It is well established that if, after the interruption of the vasoconstrictor pathways to an extremity, that extremity is exposed to a cold environment, the skin temperature of the blocked limb tends to be higher than the unblocked limb; at least  $2.5^{\circ}\text{C}$ . higher.<sup>2</sup> The skin temperature of the unblocked limb, however, tends to approach the cool environmental temperature. In our series we tried to determine how long this temperature difference was maintained, and, as mentioned before, as long as a significant difference in temperature existed in the two limbs at corresponding areas, vasodilatation as a result of the block was considered to be present.

**Results. A. Vasodilation.** Group I (6 cases). Two per cent novocaine, 5% novocaine in sweet almond oil; 1 cc. of 100% alcohol: One patient showed no effect, but 5 of the 6 patients showed an immediate vasodilatation; 2 for 3 days, 1 for 10 days, 1 for 2 weeks, and 1 till the present writing, 2 years.

Group II (4 cases). Two per cent novocaine, alcohol 1 cc.: Two cases showed no immediate effect, but 1 of these showed some vasodilatation 3 days later which was temporary. Two cases showed an immediate effect which lasted 10 and 24 days respectively.

Group III (6 cases). Two per cent novocaine with alcohol 3 cc.: Three cases showed no immediate result, but did show vasodilatation later; one 7 days later, another showed the first effect 14 days later and has continued till the present (11 months); the third showed a slight effect in 4 days and still continues to show vasodilatation at present (18 months). The other 3 cases showed an immediate full vasodilatation and lasted 10 days and from 109 to 182 days respectively. In other words, all patients in this series showed some effect, 2 showing an effect 11 and 18 months respectively. So that Group III patients gave the largest number of successful vasodilatations, but 1 case in Group I showed a remarkable result with only 1 cc. of alcohol. Summarizing the total vasodilatation effect in 14 successful blocks, 5 cases showed complete immediate vasodilatation, lasting 3 to 108 days, 9 cases showed an immediate significant or delayed significant vasodilatation lasting 3 days to 2 years.

In our past studies we have noted the difficulty in producing complete peripheral vasodilatation at room temperature below  $21^{\circ}\text{C}$ . We thought it therefore advisable to check our cases and see if any immediate results had any relationship to the room temperature in which the block was done. In no case did the room temperature ever vary more than  $2.2^{\circ}$ , although in most cases it was fairly constant. The following are the results: The 5 cases showing complete immediate

vasodilatation were done at a room temperature of 19.6° to 26.4° C.; the 7 cases showing an incomplete but significant difference in temperature were done at room temperatures of 18.6° to 26.6° C.; those showing no immediate effect were done at room temperatures of 20.6° to 26.1° C. So that the room temperature at the time of doing the block was no factor in the vasodilatation effect.

**B. Neuritis.** Group I (6 cases). Two per cent novocaine, novocaine in oil, alcohol 1 cc.: Two patients had neuritis lasting 18 and 28 days respectively. One had pain in the gluteal region, and the other had pain in the gluteal region radiating to the inguinal region and the knee. One case had local pain for 10 days.

Group II (4 cases). Two per cent novocaine, alcohol 1 cc.: There were no instances of neuritis, but 2 patients had local pain lasting 3 and 4 days respectively.

Group III (6 cases). Two per cent novocaine, alcohol 3 cc.: Four cases had neuritis. One was not followed. The neuritis in the remaining 3 lasted 10 days, 14 days and 45 days respectively. The neuritis consisted of "electric shocks" in the anterior thigh, pain in the abdomen and thigh, and pain in the right testis.

Novocaine in sweet almond oil, therefore, had no effect in avoiding painful neuritis. It seemed also that the use of larger amounts of alcohol tended to produce neuritis more often, although in all cases followed, the neuritis was of relatively short duration.

**C. Claudication Time** (Graded 0 to 4+).<sup>2</sup> Claudication time was evaluated in 10 of the 12 cases of peripheral vascular disease, and the patients were followed for 12 to 24 months. Five showed improvement, 3 showed no change in claudication status, and 2 were definitely worse, complaining that pain set in earlier than it did before the block. Study of the relation between the claudication time and the result of the paravertebral block showed, that the 2 patients who had become worse after the block, had shown no vasodilatation at any time. However, there was no definite correlation between the improvement in claudication time and either the degree or the duration of the vasodilatation.

**Comment.** There are several points which deserve mention in consideration of alcohol paravertebral block.

**I. Technique.** It is seen that in many cases, while there is an immediate vasodilatation, this effect disappears after 3 or 4 days. White's

has pointed out, that when novocaine is injected, it tends to diffuse through the tissues, and so reach the ganglia or sympathetic nerve fibers, even though the point of the needle is not directly in contact with the ganglia. Under these circumstances, when the alcohol is injected, it will not have the permanent destructive effect at the desired spot, and, although there is an immediate effect, the benefit is not lasting. One can only hope that, with skill and experience, the area sought for may be reached more often.

**II. Neuritis.** It may also be seen that novocaine in oil prior to the injection of alcohol does not prevent the occurrence of neuritis; that



neuritis occurs most often when larger amounts of alcohol are injected; and that neuritis, when it does occur, is not too severe, does not last very long, and should not be a deterrent to alcohol paravertebral block, when such a procedure is indicated.

III. *Claudication Time.* In a few patients (8) studied who showed vasodilatation, improvement or non-improvement of claudication time was not related to the degree or duration of the vasodilatation.

IV. *Degree and Duration of Vasodilatation.* Full vasodilatation as a result of paravertebral block occurred in 31% of the patients, immediately after injection of novocain. The injection of alcohol allowed this vasodilatation to be more lasting than if novocaine were used alone, but in no instance was the degree of vasodilatation as great after a few days had passed. However, 14 of the 16 patients showed vasodilatation effects lasting 3 days to 2 years. The amount of alcohol used was not the factor that determined how long this vasodilatation lasted, because the longest and best effect occurred in 1 patient who received only 1 cc. of alcohol (2 years). However, when 3 cc. of alcohol were used, there was uniform success in producing vasodilatations. Although the period of study covered only 2 years and showed that vasodilatation can last this long, there is reason to believe that the effects will last longer, and certainly long enough to produce beneficial results in the treatment of thrombo-angitis obliterans and arteriosclerosis obliterans.

Conclusion. 1. The use of 100% alcohol in lumbar paravertebral block is of definite value in producing peripheral vasodilatation. This vasodilatation may be complete, and may last for varying periods of time, even up to 2 years and perhaps longer.

2. The neuritis that is produced as a result of such a procedure occurs more often when larger amounts of alcohol are injected; it is not too severe and in no instance has lasted more than 45 days.

3. The use of novocaine in sweet almond oil did not reduce the incidence of neuritis.

4. Vasodilatation occurred more often when larger amounts of alcohol were used, but the amount of alcohol was not the factor that determined how long this vasodilatation lasted.

5. There was no correlation between claudication time and the degree or duration of vasodilatation.

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# AMEBIASIS: ANALYTICAL STUDY OF THE CASES ADMITTED TO A PHILADELPHIA HOSPITAL DURING THE LAST 5 DECADES

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AMEBIASIS is achieving a new importance during these unfortunate years of war, since at any time it may become a menace to both the civilian population and the armed forces. The old idea that amebiasis is essentially a tropical and subtropical disease is totally fallacious, and it has been thoroughly exploded by Craig<sup>2</sup> and Faust.<sup>3</sup> It is true that the disease is more common in the tropics, where in many regions an incidence of 50 to 90% is not a rarity, but it must be remembered that an estimated incidence of close to 20% has been reported for the United States as a whole.

An increase in the incidence of the disease in the United States after the war is predicted. Many of our soldiers are certain to be infested with the parasite in the tropical theaters of war. Some will become "cyst carriers" and, as such, though themselves free of symptoms, will lead to the development of the disease and its complications in the civilian population.

The diagnosis of this disease, though it can be accomplished by a relatively simple laboratory procedure, is not always easy. It depends on the demonstration in the feces of the parasite in its cystic or vegetative form. Unless the disease is suspected on the basis of an unexplained diarrhea or because of some complication, such a study often is not undertaken. Furthermore, unless a trained parasitologist is available, the organisms may not be recognized.

Goldburgh<sup>4</sup> in 1936 reported that among 232,100 admissions to the Philadelphia General Hospital from 1926 to 1935 only 1 was diagnosed as amebic dysentery. The admissions to the Jefferson Hospital during this same period amounted to 120,000, of which but 6 were diagnosed as amebic dysentery. These 6 cases were traced back to the Chicago epidemic in 1933. The same author reports that up to 1935 the Bureau of Health of Philadelphia had recorded only 43 cases of the condition, all of which occurred after 1933. In a careful survey to determine the incidence of protozoal infestation among patients admitted to the Hospital of the University of Pennsylvania during the winter of 1932-1933, Hinchshaw and Showers<sup>5</sup> reported that probably 1.9% were suffering from amebiasis. In 1934 the stools of 1040 freshman stu-

dents from a professional school in Philadelphia were carefully examined, and 4.1% were reported as positive for *E. histolytica*. In 1937 Arnett<sup>1</sup> found among 293 fecal examinations on food handlers in a Philadelphia institution, that 6.5% were positive for the parasite. He also reported an incidence of 11.3% of infestation with *E. histolytica* among the inmates and employees of an institution for the aged in the same city. He concluded that 5 to 10% of the population in this area harbored the parasite. We were unable to find definite information as to the incidence of the disease in the whole state of Pennsylvania.

We are now presenting an analysis of the cases of diagnosed amebiasis in the Hospital of the University of Pennsylvania during the last 50 years.

**Analysis of Data.** During the last 5 decades, among approximately 61,574 admissions to the various medical services of the Hospital of the University of Pennsylvania, 32 cases were diagnosed as amebic dysentery, 4 of which were complicated with hepatic abscess. Of 4764 deaths among the medical admissions of this period, only 1 was regarded as due to amebic dysentery. Out of 122,933 surgical admissions with 5233 deaths, only 1 was due to amebic hepatic abscess.

Twenty-five of the records were complete and serve as the basis of this report; the rest, although they relate to cases of amebiasis by demonstration of the *E. histolytica* in the stools, have been discarded because insufficient data are available.

Sixteen of the patients acquired the disease prior to 1933, while 9 contracted it after that year. Twelve of those who contracted the disease prior to the Chicago epidemic had been in a foreign country when they noticed their first symptoms. Thus, there were only 4 cases prior to 1933 that were infected in the United States, and of these, 1 had been in Chicago in 1924 where he was taken ill with diarrhea, while 1 had acquired the disease in New York City and 1 in Georgia. Of the 9 cases admitted after 1933, 2 acquired the infestation during the Chicago World's Fair, and one in Poland. Thus, at most only 7, 1 before and 6 after 1933, apparently acquired the disease in Pennsylvania or nearby, and 5 of these were admitted during the last 6 months.

Residence in an infested area was obviously a great aid in the diagnosis of the earlier cases, while native Pennsylvanians were not often suspected. Thus, more than half (13) of the 25 cases had been in a foreign country, either the Philippines, Hawaii, Greece, Italy, Russia, Rumania, Poland, England, France, Costa Rica, Brazil, China or Mexico; the 5 diagnosed during the last 6 months, however, have not traveled outside the state of Pennsylvania.

The ages of the patients varied from 11 to 60 years. Fourteen were between 21 and 40 years old and 6 were 40 years or older, while 5 were in the first two decades of life. Twenty-two were males; 3, females, thus giving a sex ratio of 7 to 1. Twenty-two were white and 3 colored. All 4 of those with an hepatic abscess were males, 2 of whom were colored.

The patients had various occupations, being students, laborers, food handlers, soldiers, sailors, librarians, accountants or lawyers, and one was a gypsy.

Eleven gave a definite history of seasonal onset. Four had the initial symptoms during the fall, 2 during the spring, 3 during the summer and 2 during the winter. The onset was acute in 14 and insidious in 11. Sixteen were admitted during the winter, 5 during the summer, and 4 during the fall. The duration of the disease before hospitalization varied from 3 months to 17 years (average, 4.3 years). Only 7 patients were admitted within the first year of the onset of their symptoms.

**Symptomatology and Physical Observations.** As shown in Table I the most prominent symptom in the patients studied was diarrhea, 24 of the patients having at some time a severe diarrhea with 4 to 20 movements daily. In all of these, remissions and exacerbations were the rule. The remaining symptoms most commonly complained of were: blood in the stools, mucus in the stools, abdominal pain, rectal tenesmus, dyspepsia, fever, loss in weight, headache, chills and jaundice. The most important physical finding was abdominal tenderness, observed in 19 of the cases. Hepatomegaly was recorded in 8 cases and it was due to hepatic abscess in 3 of these. Mild jaundice was observed in 2 of the 4 patients suffering from hepatic abscess.

TABLE I.—SYMPTOMS AND SIGNS OF 25 CASES OF AMEBIASIS

%	No. of cases		Total
96	24	Diarrhea	24
96	24	Remissions and exacerbations	24
84	21	Bloody stools	21
80	20	Pain in abdomen	20
76	19	Mucus in stools	19
76	19	Abdominal tenderness	19
68	17	Tenesmus	17
68	17	Fever	17
68	17	Loss of weight	17
68	17	Hepatomegaly	17
32	8	Nausea and vomiting	8
28	7	Anorexia	7
28	7	Headache	7
20	5	Chills	5
16	4	Jaundice	4
8	2	Constipation	2
4	1		1

**Laboratory Observations.** In 22 cases vegetative forms of *E. histolytica* were found in the feces; in 2, the cystic forms. In 1, motile amebæ were found in the pus draining from an hepatic abscess. In all of the most recent cases the diagnosis was confirmed by repetition of the stool examinations. Among 14 cases submitted to proctoscopic examination, 10 had ulcerative rectosigmoiditis; 2 presented catarrhal inflammation and in 2 the mucosa was normal. Eleven patients showed anemia. Anemia was severe in a patient with thrombocytopenic purpura hemorrhagica who had become infested with *E. histolytica* some 3 years previous to admission. Eleven cases had a leuko-

cytosis. Eosinophilia was encountered in 9 instances, ranging from 9 to 12%. No other ova or parasites were seen in the feces in any case. Treatment. The treatment consisted of colonic irrigations of plain water at a temperature of 43° to 45° C. in 6 of the cases. Warm irrigations of mercurochrome, quinine and silver salts were given to 3 patients. Kerosene irrigations were prescribed in 1 instance. Emetine, including carbarsone, stovarsol and chiniofon in 16 cases. Surgical drainage and emetine hydrochloride were employed in the treatment of the 4 cases suffering from hepatic abscess. In the majority of the patients the treatment was inadequate by present standards. Two patients died (8%), both from complications; hepatic abscess in one and perforation of the bowel in the other. Autopsy was not permitted. Twenty-two of the patients were discharged as improved, and 1 is at present under observation but recovering rapidly. Due to lack of proper follow-up the true percentage of cures cannot be reported with accuracy, especially in the cases admitted previous to 1933. Below we present abstracts of case histories of 3 patients, 1 showing a typical abscess, 1 with an unusual type of abscess and 1 of the patients who died.

**Case Studies.** Case 1. B.B., a 32 year old negro, was admitted to this hospital on January 20, 1938. He had been well until 9 days previously when he developed pain in the epigastric and presternal regions which was intensified by deep breathing. He had had frequent bouts of diarrhea after traveling in South America in the years 1930-1932. He was hospitalized in the Boston City Hospital in 1935 because of bloody stools and right upper quadrant pain, with diagnosis of chronic cholecystitis and toxic hepatitis. A cholecystotomy was performed at that time.

On this admission examination revealed a temperature of 102.5° F., pulse of 114 and blood pressure of 118 systolic and 82 diastolic. He was slightly jaundiced and breathing was rapid and shallow. He complained of severe stabbing pain in the right upper quadrant and over the hepatic area there was exquisite tenderness. There was definite diaphragmatic lag on the right side. His leukocyte count was 21,200, with a normal differential; the red blood cells numbered 3.54 millions, with a hemoglobin of 68%. The van den Bergh test was immediate direct and the indirect reading was 4 units. Urine was positive for bilirubin and bile salts. The Kolmer and Kahn tests were weakly positive. The stool examination was positive for *E. histolytica* trophozoites 2 days after admission.

Three days after admission surgical exploration revealed an abscess the size of a hen's egg close to the diaphragm in the mid-zone of the liver anteriorly. Treatment consisted of drainage and emetine hydrochloride subcutaneously. He was discharged cured.

Case 2. C.J., a 46 year old negro, admitted to the Hospital of the University of Pennsylvania on November 25, 1941, was well until 18 months previous to admission when he developed a bloody diarrhea and tenesmus. Two weeks before admission he started to complain of headache, chills, fever, dizziness and pain in the right upper quadrant. He had been in foreign countries while in the Army and Navy, but for the past 5 years had been a resident of Philadelphia.

On examination his temperature was 101° F., pulse 100, and respiration 24. Dullness, increased tactile fremitus and fine rales were encountered at the base of the right lung. There was marked tenderness and rigidity in the right upper quadrant. His leukocyte count was 21,000 and the hemoglobin was 65%. A chest plate demonstrated increased bronchial markings suggestive of bronchiectasis.

The day following admission an exploratory laparotomy was done and an acute catarrhal inflammation of the gall bladder was encountered. A cholecystotomy was performed. The draining bile appeared normal, and drainage continued for a few days. This gradually faded in color and decreased in amount; about the 10th postoperative day he began to drain frank pus. The right upper quadrant pain recurred, the liver became markedly enlarged and on the 24th postoperative day drainage became profuse and reddish in color. Two days later the incision was enlarged and an enormous liver abscess was drained. This had perforated spontaneously into the gall-bladder and had partially drained through the tube left *in situ* at the first surgical intervention. Examination of the pus revealed motile *E. histolytica* trophozoites. Following emetine hydrochloride the parasites were eradicated from the draining pus and they were not at any time found in the feces. He was discharged 2 months after admission, but the patient was subsequently readmitted to the hospital for a recurrence of the diarrhea, blood in stool, and tenesmus. Intensive treatment with emetine, chiniofon, and carbarsone, however, rendered the feces free of amebae and the patient was discharged as cured.

CASE 3. C.S., a 14 year old white female, was admitted to this hospital on November 16, 1922, complaining of intermittent attacks of bloody diarrhea for 3 years. Two years previously she was told that she had amebic dysentery and was treated with emetine for 3 weeks. Subsequently she received emetine and ipsecac sporadically, but during the 9 months previous to her admission such medication was of no avail.

Examination showed that she was cachectic. Red blood cells numbered 3.9 millions, with a hemoglobin of 65%. Her leukocyte count was 16,200 with an eosinophilia of 10%. The stools were grossly bloody and motile forms of *E. histolytica* were seen. Proctoscopic examination revealed diffuse ulceration of the mucosa of the rectum and sigmoid.

The patient's course was steadily downhill. She had as many as 20 bowel movements a day terminally. She developed a low-grade fever, and in spite of colonic irrigations, emetine hydrochloride, kaolin, paregoric, bismuth sub-carbonate, transfusions and general supportive measures, she died 30 days after admission.

**Comment.** It has been demonstrated that amebiasis is a serious problem in any locality, and that the city of Philadelphia is no exception. Nevertheless, during the past 5 decades only 32 cases of this disease have been diagnosed and treated in this hospital. Thus, only about 0.05% of all medical admissions were cases of amebiasis. This, undoubtedly, is a small percentage when one considers that the incidence of infestation in special groups in the city has been shown to be approximately 5 to 10%. Furthermore, only 7 cases were known to have acquired the disease in Philadelphia or nearby, perhaps indicating that the index of suspicion in this region is low. Undoubtedly, the tendency has been to make stool examinations in those patients who traveled in the tropics because amebiasis abounds there. We believe that many cases were probably missed because not enough stools were examined. A stool examination is not a routine procedure in many hospitals in the United States, but at least in all cases of diarrhea such studies should be required.

There was only one patient listed in our files who could be classified as asymptomatic at the time of admission. Nevertheless, he gave a history of traveling in South America, and besides stated that he had had relapses and exacerbations of bloody diarrhea. Of the 32 cases admitted to this hospital during the last 50 years, 5 were diagnosed

and treated during the last 6 months. It seems likely that intensified interest in the disease has been responsible for the increase in the number of cases diagnosed recently.

Evaluation in the treatment of amebiasis is apparent in this series of cases. In the last few years treatment standards have improved considerably. Present-day medical therapy consisting of intensive courses of emetine, chiniofon and carbarsone has been shown to be more effective than former therapeutic regimens, and liver abscesses are better managed by simple aspiration and emetine rather than by the now obsolete, open operation.

**Conclusion.** An increase in the incidence of amebiasis is to be expected in the United States after the war, as the disease, no doubt, will be brought into this country by service men returning from the tropics. Epidemics may arise from chronic "cyst carriers."

Although the incidence of the disease in Philadelphia has been estimated to be 5 to 10%, only 32 cases have been diagnosed at the Hospital of the University of Pennsylvania during the last 5 decades. Five of these cases have been diagnosed during the last 6 months. Until recently, interest in amebiasis in this locality has been at low ebb. Complications of the disease are not uncommon, as evidenced in this series of 25 cases. A high index of suspicion and a competent parasitologist to do stool examinations are necessary prerequisites for proper diagnosis.

We are indebted to Dr. T. Crier Miller for his assistance in the preparation of this report.

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## TRICHINELLA SKIN TESTS IN PATIENTS IN GENERAL HOSPITALS AND TUBERCULOSIS SANATORIA\*

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The study of routine autopsies has revealed a great variation in the incidence of trichinella infestation in different parts of the United States. The trichinella antigen and control solutions in this study were supplied by Lederle Laboratories, Pearl River, N. Y.

States. In Rochester, N. Y., the incidence was reported to be 17.2%, in Boston 27.6%, in San Francisco 24%, in Minneapolis 17.1%, in New Orleans 3.5% and in St. Louis 15.5%.<sup>5</sup> In a study of 105 autopsies in North Carolina, 3 (2.8%) instances of trichinosis were found. The diaphragms from these patients were examined by both the muscle press and digestion technique.<sup>5</sup> The present study was undertaken to compare the percentage of cases found to have evidence of infestation as determined by positive intradermal reactions with the previously determined incidence at postmortem examination.

Kaljus<sup>6</sup> points out that Fülleborn first proposed the idea of a skin test for the diagnosis of trichinosis, but he was unable to isolate the parasites in the pure state. A few years later Bachman developed a method for obtaining isolated trichinae and, by a process of drying and extracting, obtained an antigen which gave a positive skin test in infected rabbits and guinea pigs.<sup>6</sup> An antigen prepared by the Bachman technique was first used in man by Augustine and Teller.<sup>6</sup>

In 1937, Spink<sup>7</sup> noted that two types of reaction occur in humans: an immediate reaction with blanched wheal, occasional pseudopods and (reticulated) erythema which appears within 5 minutes, and a delayed reaction with (solid) erythema and edema which does not become positive for 12 or more hours and is read 24 hours after the test is given. The immediate reaction is not obtained until the 2d week of infection, usually on or after the 17th day. The delayed response is elicited only early in the disease.

Since that time numerous reports have appeared of the use of the intradermal reaction in the diagnosis of trichinosis. McCoy, Miller and Friedlander<sup>7</sup> used 1:10,000 and 1:500 dilutions of antigen. In early known infections of 6 weeks or less, immediate reactions were obtained in 70% with the 1:10,000 dilution; in late infections of 3 to 22 months duration 49%, and in infections of 3 to 7 years duration 23% were positive. A control series showed 9% positive reactions in Rochester and 4% in San Francisco in individuals tested at random; 18% of patients in Louisiana infected with *Trichinuris trichiura* gave positive tests. Schapiro, Crosby and Sickler<sup>8</sup> found an incidence of 18.3% in 400 unselected individuals in Washington, D. C. Gould<sup>9</sup> found 5.9% positive reactions in 2940 patients in Detroit.

Recently, several studies have been made which correlate the incidence of trichinosis found at autopsy with that found by routine skin test. Schapiro *et al.*<sup>8</sup> reported 116 patients who were skin-tested during life and examined for trichinella larvae at postmortem. Larvae were found in 26 of the patients of whom 24 had given positive immediate reactions (92.3%); the other 2 patients had light long-standing infections. No larvae were found in 90 of whom 3 had given (false positive?) immediate reactions (3.3%). Gould<sup>9</sup> examined 388 patients at autopsy for trichinella larvae, all of whom had been skin-tested during life. Using a 1:10,000 antigen, he found that only 10.1% of the patients who were found to have trichinella larvae at autopsy had given a positive immediate intradermal reaction and in those in whom no trichinae were found immediate (false positive?) reactions were



In view of the great variation in the incidence found at autopsy and findings was attributed to mild, long-standing subclinical infections. The failure of correlation of clinical and autopsy does find both living and dead larvae. The skin test should not have these limitations, since it may be positive regardless of whether the infestation is light or heavy, or the larvae dead or alive.

**Material.** A total of 700 patients were skin-tested; 278 consecutive admissions to the North Carolina Baptist Hospital, 144 patients at the Forsyth County Hospital, 120 patients at the Guilford County Tuberculosis Sanatorium, and 158 patients at the Western North Carolina Tuberculosis Sanatorium. Of the patients, 522 were white and 178 negroes; 315 were males and 385 females. The youngest patient was 9 years of age, the oldest 111. In 558 of the patients, the presence or absence of a history of an allergic condition was noted. Seventeen individuals gave a history of allergy; 9 had attacks of asthma, 2 of allergic dermatitis, 2 of urticaria, 2 of hay fever, 1 of hay fever and asthma, and 1 patient had asthma, hay fever, migraine, and allergic dermatitis.

**Method.** In all instances a 1:10,000 trichinella extract and a saline control solution were used. An area on the flexor surfaces of both forearms was cleaned with alcohol and 0.02 to 0.03 cc. of the test and control solutions were given intradermally in separate arms. The reactions were read 15 to 20 minutes and 24 hours later; the degree of erythema and wheal and the presence or absence of pseudopods were noted. The reaction was recorded as positive when the diameter of the area of erythema with induration exceeded that of the injected bleb by 5 mm., or when the wheal exceeded the diameter of the injected bleb by 3 mm. If the control injection produced a reaction, the test was recorded as negative.

New syringes and needles were obtained for the skin tests and were not used for any other purpose. One syringe and needle was always used for the test solution and another for the control; the syringes and needles were never interchanged. After being used they were washed with distilled water, placed in glass tubes, and sterilized in an autoclave.

**Results.** Seventy of the 700 patients (10%) gave positive tests. The incidence at the Baptist Hospital was 5.8%, at the Forsyth County Hospital 10.4%, at the Guilford County Tuberculosis Sanatorium 16.9%, and at the Western North Carolina Tuberculosis Sanatorium 13.3%. Of the 70 positive tests, 68 were immediate reactions; in 10 patients the positive reaction persisted for 24 hours. In 2 patients the reaction was delayed in type. The incidence among the white patients was 9.1%; among the negroes 11.8%. The patients who had spent most of their lives in a community with a population of 2500 or less were classified as rural inhabitants and showed 9.8% positive reactions; those living in larger communities, the urban group, showed 10.3% positive reactions. The incidence in males was 11.1%, and in females 8.8%. Tuberculosis was present in 283 patients and 14.3% gave positive reactions; the incidence in those without known or clinical tuberculosis was 7.1%. Among the patients

with tuberculosis 17.2% were negroes; 30% of those without tuberculosis were negroes. Table 1 summarizes the findings.

TABLE 1.—RESULTS OF 700 SKIN TESTS WITH TRICHINELLA ANTIGEN 1:10,000

[illegible]

Five (29.4%) of the allergic patients were positive to both the test and control solutions and were counted as negative; 10 (58.8%) had a negative test and control, hence only 2 (11.8%) were considered to have a positive reaction. One patient had a typical strongly positive test while acutely ill with asthma and dermatitis, and again when re-tested after recovery from the attack. One patient with recognized hookworm infestation gave a positive test; repeated tests were positive 1 week and 6 weeks after anthelmintic therapy. One and 6 weeks after treatment, which resulted in the passage of parasites, stool examinations were negative for ova and parasites. Stool examinations were not done on all patients; this one instance was thought to be a coincidental infestation.

None of the patients with positive reactions gave a history which suggested acute trichinosis, although the majority consumed fairly large amounts of pork which may not always have been well cooked. No untoward systemic or local reactions to the test were observed. The incidence of infestation as determined by intra-

dermal test (10%) is definitely higher than that previously found here at autopsy (2.8%).<sup>5</sup> In the study of autopsies only a small portion of the total musculature is examined. The skin test should determine sensitivity to the parasites regardless of where they have localized or in what concentration. The difference in incidence reflects the relative effectiveness of the two methods in discovering past infestation. Lack of specificity of the intradermal reaction, which might account for a higher incidence by this method, has not been demonstrated when a dilution of 1:10,000 is used.<sup>6</sup> The incidence in other parts of the country is greater than that

found here, as determined by the study of autopsy material. By skin test by the same technique, the findings have varied widely. The incidence of trichinosis in grain-fed hogs is 1 to 1.5% and 4.8% in those fed garbage.<sup>1</sup> The majority of hogs in this section are grain-fed. Uncooked or prepared pork products such as blood sausage and salami are not eaten in the South as much as in the North where the incidence of the disease is higher.

The incidence was 9.8% among rural inhabitants and 10.3% among residents of towns with a population of 2500 or more. This does not support the contention that trichinosis is principally a disease of rural areas. The source of pork in North Carolina is essentially the same in rural and urban areas; the majority comes from small, local packing houses, though in the large cities western meat processed by large packers is sold.

More reactions were found in males than in females: 11.1% and 8.8% respectively. Men are more likely than women to eat away from home at roadside cafes where cooking is unreliable, and where pork products, such as barbecue and sausage are featured. Women may develop the disease while tasting sausage and other similar raw or partially cooked pork products while these foods are being prepared. The apparent increase in incidence among negroes, 12.3% against 8% in the white patients, would not be surprising, if true, since the negro tends to prepare food poorly. However, the difference is not statistically significant.\*

TABLE 2.—INCIDENCE OF POSITIVE REACTIONS TO TRICHINELLA ANTIGEN IN 700 PATIENTS ACCORDING TO AGE

Age group	Patients tested	Patients positive	% positive
0-9	1	0	0
10-19	43	5	11.6
20-29	198	21	10.6
30-39	163	15	9.2
40-49	120	9	7.5
50-59	75	11	14.7
60-69	47	4	8.5
70-79	36	3	8.3
80-89	16	1	6.3
90-99	.	.	.
100-109	.	.	.
110-119	1	1	100.0
Total	700	70	10.0

Hall,<sup>3</sup> studying the ages of the 1000 persons whose diaphragms were examined by Nolan and Bozicevich, found an incidence of 16% in persons up to 50 years of age, and 19.5% in those over 50. In the study of muscle removed at autopsy, Gould<sup>2</sup> also found a greater incidence with increasing age which he attributed to the greater number of opportunities for exposure to trichinae as the individual grows older. Using the intradermal test he found a slight decrease in incidence with advancing age. In the present series 524 individuals were under

\* We are indebted to Dr. Donald S. Martin, Duke University School of Medicine, Durham, N. C., for statistical criticism of our results.

50 years of age, of whom 50 (9.5%) gave positive reactions; 176 were over 50 years of age, and 20 (11.4%) gave positive reactions. No gradual increase in incidence with advancing age was noted (see Table 2); the difference in incidence among the patients under and over 50 is of doubtful significance. Some individuals over 50 years of age, who had trichinosis when young, might have lost their sensitivity and hence ability to give a positive skin reaction. It has been reported that in some instances the skin test remained positive for 7 years after infection, while in others a negative test was found 4 years after the illness.<sup>7</sup>

Arbesman, Witelsky and Osgood<sup>1</sup> have found that allergic individuals show a higher incidence of positive reactions than do non-allergic subjects. Others believe that the allergic state predisposes to false positive non-specific reactions.<sup>7</sup> This was not the finding of Gould.<sup>2</sup> The incidence among 17 white allergic patients of the present series was 11.8%. This is higher than the incidence found among the entire group of white patients (8%), but since the number is small, is probably of no significance. Allergic individuals have an hyperactive skin, which one would expect to react to the control as readily as to the test solution; 5 of our allergic patients reacted to both, hence were considered negative in the statistical analysis.

The rise in incidence among patients with tuberculosis is striking (Table 1) and, so far as we have been able to determine, has not been previously reported. The difference is statistically significant. The increase in positive reactions was noted in both negroes and whites with tuberculosis. Although the incidence is higher in all negroes than in whites, the number of whites with tuberculosis was far greater than of negroes, hence this factor was not significant. Since the syringes and needles used in this study were never used for any other purpose, and were never sterilized by boiling, no chance contamination with tuberculin occurred. The larvae from which the antigen was prepared were recovered from rabbits which had never received injections of tuberculin, nor was tuberculin ever used or made in the laboratory where the antigen was manufactured.

Almost all of the patients with tuberculosis were in 2 sanatoria; it is possible that these findings represent the result of 2 local, unrecognized or subclinical epidemics. In 1 sanatorium the hogs were garbaged, and reared on the grounds; in the other the pork was purchased, for the most part, from large packers. In any institution where the quantity used is greater than in a private home, pork is frequently less thoroughly cooked. The size of the uncut portions cooked is larger and the center may be inadequately cooked even though the surface is thoroughly done. Of the 144 patients at the Forsyth County Hospital, 87 were confined to the chronic or mental wards and had resided in an institution for a period of time comparable to the stay of patients in a sanatorium. Of these, only 7 (8%) gave positive tests, suggesting that confinement or residence in an institution is not the sole explanation for the rise in the tuberculous group. The remainder of the patients at the Forsyth County Hospital and those

at the Baptist Hospital constitute the group in general hospitals where the stay in an institution is too short to be significant.

Other possible explanations are that patients with tuberculosis develop skin sensitivity with much lighter infestations or retain sensitivity longer than do non-tuberculous individuals, or that their skin is more reactive to intradermal tests in general. Personal communications from men with experience in treating tuberculosis indicate that, in general, they have not found their patients more reactive to intradermal tests than non-tuberculous individuals. It seems unlikely that these patients would develop a response to trichinella antigen and not to other materials commonly used for intradermal tests, for many patients had been used previously for passive transfer tests in the study of allergic conditions without evidence of unusual reactivity.

**Summary.** 1. The incidence of positive intradermal reactions to commercial trichinella antigen in North Carolina, was 10% in a series of 700 hospitalized patients. This is lower than similar studies have shown in some other areas of the United States.

2. The incidence found by skin test was greater than that found in routine autopsies in this geographic area.

3. The incidence was greater among negroes and in males, but was not statistically significant in either.

4. The incidence of positive tests was essentially the same in urban and rural groups.

5. Patients with active tuberculosis in 2 sanatoria gave a higher percentage of positive reactions (14.3%) than did those without tuberculosis (7.1%) in 2 general hospitals. This was found to be statistically significant.

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## HEART BLOCK

## A STUDY OF 100 CASES WITH PROLONGED P-R INTERVAL

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In a study of 6732 electrocardiograms taken on 4264 patients at the Lawson General Hospital, there were 100 instances of first degree heart

block, with P-R intervals of 0.22 second or above. There were 12 additional instances with P-R intervals of 0.21 second which were omitted from this study since certain authors<sup>1</sup> feel that a P-R interval of 0.21 second is normal.

This study includes individuals observed in the Cardiovascular Section from July, 1941 to August, 1943. It represents patients treated in the Cardiology Wards as well as those observed in other sections and departments of the hospital on whom electrocardiograms and consultations were requested. Ninety per cent of the total number of patients observed had no symptoms or clinical evidence of heart disease and were seen because of routine requirements. The remaining 10% had evidence of heart disease. Of the 100 cases selected for this study, 91 cases had a complete cardiovascular survey, including Roentgen ray studies of the heart and lungs. The remaining 9 cases did not have cardiac consultations but did have electrocardiograms and complete physical examinations as well as Roentgen ray studies of the heart and lungs.

**Etiologic Classification of Cases.** In the 2-year period over which our observations extended, the incidence of heart block was 2.3% in the 4264 patients studied. The etiologic classification of the 100 cases is given in Table 1.

TABLE 1.—ASSOCIATED DISEASES IN 100 CASES OF FIRST DEGREE HEART BLOCK

1. Rheumatoid arthritis . . . . .	5
2. Arteriosclerosis, coronary . . . . .	12
3. Arteriosclerosis, generalized . . . . .	2
4. Digitalis . . . . .	2
5. Gonorrhea . . . . .	8
6. Hypertension . . . . .	1
7. Infectious diseases . . . . .	5
8. Miscellaneous . . . . .	11
9. Neurocirculatory asthenia . . . . .	7
10. No disease found . . . . .	19
11. Rheumatic fever . . . . .	28
Total . . . . .	100

In the arthritic group, there were 5 cases of rheumatoid arthritis. These findings are of interest because of recent investigations<sup>1</sup> in which rheumatic-like lesions have been found in hearts of people at autopsy who suffered with rheumatoid arthritis.

Of the 12 cases of coronary arteriosclerosis, 8 cases had myocardial infarction and 4 cases had angina pectoris. Of the 2 cases in which generalized arteriosclerosis was the only causative factor of block, no other evidence of disease of the cardiovascular system could be determined.

There were 2 cases in which digitalis therapy was considered to be the cause of the prolonged P-R interval. There were 8 cases of heart block found among 530 cases of sulfonamide-resistant gonorrhea, examined prior to the administration of some sulfonamide drug 2 to 3 weeks before cardiac consultation. This represents an incidence of 1.5%. The cause of the heart block in this

group is not clear. It is felt that the block was not due to the sulfonamide drugs. The incidence of block in these cases compares favorably with the reported incidence of 1.5% of block<sup>3</sup> in a study of 1812 normal males who had P-R intervals above 0.20 and who had not received any type of drug therapy.

There was 1 case of hypertension; the blood pressure was 180 systolic and 100 diastolic. In the group with infectious diseases, there was 1 case each of malaria, German measles and infectious mononucleosis. One patient had a history of scarlet fever and another a history of diphtheria during childhood. During the acute illness, the patient with German measles developed paroxysmal, complete heart block for a period of 24 hours. There was a subsequent return to normal of the P-R interval over a period of 1 week.

The miscellaneous group included such causative factors as spontaneous pneumothorax, mediastinal lymphoblastoma, congenital heart disease, acute and chronic nephritis, migraine, psychosis and tuberculosis pericarditis. One patient had a past history of thyroidectomy in 1932 and another a history of syphilis in 1923.

There were 7 cases of neurocirculatory asthenia in which no evidence of organic heart disease could be determined. Under the classification of no disease found, 19 patients were studied in whom, except for the prolonged P-R interval, no other evidence of disease of the cardiovascular system could be demonstrated. This group consisted of the following: 6 civilians who were candidates for commission, 2 instances of psychoneurosis, 2 of fractures and 1 instance each of psychosis, involuntal melancholia, pylorospasm, obesity, upper respiratory infection, inguinal hernia, duodenal ulcer, traumatic hematemesis and 1 patient who was hospitalized for determination of physical fitness. There was no history or evidence of any disease that might reasonably be expected to be associated with a prolonged conduction time.

In the rheumatic fever group, there were 22 cases of acute rheumatic fever and 6 cases of chronic rheumatic infection. Of the entire group, 9 cases had chronic valvular heart disease, one of which gave no history of previous rheumatic infection. Four patients had acute pericarditis. Of the entire series of 100 cases with block, 34% fell into the category in which no plausible explanation could be found for the prolongation of the P-R interval. This group was composed of the 19 cases in which no disease was found, the 7 cases of neurocirculatory asthenia and the 8 cases of gonorrhea.

Comment. The P-R intervals of the 100 cases are tabulated in Table 2. In 79 cases, the interval varied from 0.22 to 0.24 second; in 21 cases, the interval varied from 0.26 to 0.44 second. The variations in age and pulse rate are grouped according to the etiologic classification of the block.

In our series, the greatest incidence of block was in the 28 cases in which rheumatic fever was the causative factor. The next largest





with the act of swallowing. In both cases, the block was relieved by atropine.

To study the effect of atropine on conduction time, doses of 1/75 grain were given intravenously in 38 cases of this series. In 25 cases, the P-R interval returned to normal, while in 13 cases, there was no change. Of the 38 cases in which atropine was used, 25 cases (65%) showed a return to a normal conduction time of 0.20 second or below. Those responding included 7 cases of rheumatic fever, 4 cases of gonorrhea, 5 cases in which no disease was found, 3 cases of neurocirculatory asthenia, 2 cases of sore throat, 2 cases of rheumatoid arthritis, 1 case of acute nephritis and 1 case of generalized arteriosclerosis. Of those not responding, there were 6 cases with rheumatic fever, 4 cases in which no disease was found, and 1 case each of neurocirculatory asthenia, gonorrhea and congenital heart disease.

We feel that the return to a normal conduction time, following the administration of atropine, indicates the block to be of vagal origin. However, the disappearance of auriculo-ventricular heart block after the use of atropine, does not mean that there may not be a pathologic change in the heart muscle or conduction system causing the block. It is not uncommon for heart block, which occurs in acute rheumatic fever and other acute infections, to be relieved by atropine. It is possible that in such cases there may be some change of vagal tone through altered physiology at the myoneural junction. Carter and Dieulaide<sup>2</sup> reported a case of recurrent complete heart block in which the P-R interval returned to normal with atropine, yet necropsy showed a badly diseased bundle.

**Summary.** A series of 100 cases of heart block with prolonged P-R intervals is reported. The study consists of a review of 6732 electrocardiograms taken on 4264 patients at the Lawson General Hospital. The etiologic classification of block is given with a discussion of causative factors. The P-R intervals of the 100 cases are tabulated, as well as variations in age and pulse rates. The normal range of the P-R interval is discussed. The belief is expressed that an occasional person may have a prolonged conduction time which is normal for that person and is perhaps associated with an individual variation of vagal tone. Observations are given regarding the effects of atropine in heart block with an expression of our beliefs as well as those of other observers concerning its use.

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## NEUROPSYCHIATRIC CASUALTIES FROM GUADALCANAL

## I. PERSISTENT SYMPTOMS IN THREE CASES\*

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ALTHOUGH 15 to 20% of war casualties reaching the United States are neuropsychiatric,<sup>11</sup> the relative proportion of these cases was much higher in the Guadalcanal Campaign. Forty per cent of the total evacuated to this country from Guadalcanal suffered from disabling neuro-mental disease. Only 5% of a comparable group from Pearl Harbor were of this type.<sup>24</sup> Smith,<sup>24</sup> under the title "Guadalcanal Neurosis," described these cases as a new and unique psychiatric malady. These soldiers were reduced to a pitiable state of military ineffectiveness after prolonged exposure under severest tropical conditions to exhaustion, fear, malaria, and sudden violent death at the hands of an insidious and ruthless enemy.

Three such cases, observed 10 months after leaving Guadalcanal are presented in some detail. The dramatic severity and persistence of a combat-incurred neurosis, similarity of symptoms, and absence of psychiatric irregularity in the pre-morbid personality are features of particular significance in these cases. In this paper are considered the immediate physical and psychologic elements responsible for mental breakdown in this group of soldiers.

**Case Reports.** Case 1. Age 27, born in a small manufacturing town in Massachusetts. Father: American born, high school graduate, considered a competent and efficient worker in a shoe factory. He is irritable, high-strung and addicted to alcohol. Divorced by patient's mother, who remarried. Mother: Born in Massachusetts, of normal temperament and disposition. There are 7 siblings, all living and well. There is no history of mental disease in the immediate or remote family line.

**Personal History.** Patient was born in the same Massachusetts town as his father. At the age of 2 he had infantile paralysis and was treated at a local clinic for some years thereafter. Minor weakness and atrophy of anterior tibial group of muscles of right lower extremity persist as residuals. No disability or gait disturbance. Active indulgence in sports of all kinds, including football during childhood years. Chickenpox and whooping cough at 8. Chorea, at 9, required temporary absence from school; no residuals. Progress at school irregular because of indifference, and interest in athletics and social diversions. No history of truancy, or difficulty with school or legal authorities. Left school during 8th grade. Patient was active in social affairs, well liked, and devoted to his many friends. Work record is excellent; worked steadily and efficiently at one job in a paper mill from the time of leaving school to date of induction, earning \$32 to \$35 per week. Married shortly after induction. In every respect marital relationship has been mutually satisfactory; patient considers himself happily married; desires children after the war. Army record is excellent. Became a private first-class. "I did not try to further myself. I was satisfied to be a soldier."

**Present Illness.** Patient was landed on the Guadalcanal beachhead in October, 1942 where he participated in the campaign to extend the beachhead and to capture Henderson Field. He suffered all the privations of active bitter

\* This is the first of 2 papers on Psychiatric Casualties from Guadalcanal. A forthcoming contribution will concern a reevaluation of the predisposition factor in neuroses of war.

For a time, his position was exposed to repeated bombing raids by enemy planes and to shelling by high-caliber naval guns by elements of the enemy fleet. Despite frequent malaria chills, he was maintaining in front line fighting. Four weeks after landing at Guadalcanal, while his party was attempting to silence a Japanese machine gun nest, his position fell under direct fire of Japanese howitzers. Patient recalls distinctly that 2 shells exploded nearby almost simultaneously, one on either side of him. He was rendered unconscious, remaining so for 2 hours. He recalls, with difficulty, events of the following 2 weeks. He vaguely remembers being carried to a field hospital and then being evacuated by air to a hospital establishment on another island. Here he had an attack of malaria with severe chills and high temperature. Patient was then evacuated to a more permanent installation where, 4 weeks after his initial accident, he was seen by a psychiatrist. "I was nervous, shot to pieces and my head ached. I was mostly in a daze. I lost my memory a little part of the time. I could not remember my address." No associated nausea, vomiting or visual difficulties. Patient was then sent back to the United States. After months of rest, combined with active treatment in two general hospitals, patient was sent home on a 30-day sick leave. Patient was then put on a limited service status, with a diagnosis of psychoneurosis, anxiety type (battle reaction). He was then referred to our Training Center. Because of irritability, headache, insomnia, difficulty in concentration, tremor and inability to perform even minor military duties, patient was referred to the Psychiatric Section of the Station Hospital for observation. Examination. On admission to the hospital examination revealed a well proportioned individual of normal height and weight. Muscles were firm and well developed. Heart and lungs were normal. Abdominal examination disclosed no evidence of splenomegaly or hepatomegaly. Roentgenologic examination of chest and laboratory examinations, including numerous smears for malarial parasites, were negative. Icteric index was normal. Neurologic examination disclosed minor residuals of old poliomyelitis in the right lower extremity. There was no apparent foot drop, however. In addition, fine regular tremor of the head and outstretched hands was present in moderate degree; increased perspiration was noted, especially when patient related his Guadalcanal experiences. During interviews patient became anxious and voluble while discussing his symptoms. "It's too much for me. I get nervous when I get out there and have to give orders. I can't do it." Despite relatively recent return from a month's sick leave, patient states, "I'd like to get a leave, Sir. I'd like to go home for a while. I'd like to get an assignment closer to home. God knows when I'll get home again." Asked what assignment he felt capable of tackling, "I'd like to be a guard, Sir." Later, "I'd better take that back. You can't tell. I don't know about the gun part of it. I am afraid something might happen. I might hurt or kill someone." Patient trembled visibly. "You can never tell about fellows that went through Guadalcanal. Everything I hear makes me think of that place. I could not handle another rifle. The slightest noise makes me jump. I was once a good soldier, but not any more. I just cannot do anything. A fellow changes when he comes back from what I went through." Asked about a medical discharge, "I could use an army discharge, but I don't care." This patient, and the 2 others from Guadalcanal, were somewhat difficult to handle on the ward. The 3 were often reported for irregularity in conduct. On several occasions they were reported for failure to observe ward regulations. They were irascible, noisy, and at times frankly aggressive. Irregularities of this type, however, were by no means the rule. Otherwise, these patients were obedient, presentable and soldierly. Unpredictable and sudden change in attitude and behavior was quite striking. All attempts to reclaim this soldier failed. Physical activity of any kind left him weak and trembling, and usually gave rise to headache. Indoor assignments were no more successfully performed. Patient failed in accomplishing the most trivial military duties. In July, 1943, 9 months after leaving Guadal-

canal while under observation in the hospital, patient suffered exacerbation of malaria with chills and high temperature. Tertian type malarial parasites were identified in the blood smear.

CASE 2. Age 23, born on a small farm in Missouri. Father was a tenant farmer in impoverished circumstances, though considered normal in temperament and disposition. Both parents were born in Missouri; both literate. Mother is considered stable and dependable. There are 4 siblings, all living and well. Patient is the youngest in the family. There is no history of nervous or mental disease in the immediate or remote family line.

*Personal History.* Patient was born on father's small Missouri farm. No known serious illness or injuries as a child. Patient began school at the age of 5, reached the 8th grade; left at the age of 15. Attendance record was good and there is no history of chronic truancy or of difficulty with school authorities. Had little difficulty learning, but was not particularly interested in obtaining an education.

He was arrested once at the age of 17 for writing a check for \$6, but patient was not indicted and was released upon payment. No further difficulties with legal authorities.

Work record was fair. He worked for various neighbors on their farms and had various temporary jobs, such as truck driving during the harvest season. During these years patient lived at home with his parents. Was regarded as somewhat headstrong but otherwise was popular with boys of his own age group. No chronic social or sexual difficulties.

Married in 1940 at the age of 19, obtaining a divorce 10 months later. Separation stated to have been due to infidelity and uncongenial habits of his wife. Sexual habits were somewhat promiscuous after divorce. He contracted gonorrhea twice. Occasional overindulgence in alcohol, rarely to the point of intoxication. Army record is excellent. He has never been in difficulty with army authorities except on 1 occasion when company punishment was meted out for gambling. He was regarded as an aggressive, dependable soldier.

*Present Illness.* Patient was set ashore on the island of Guadalcanal with the first wave of troops landed in October, 1942. On the night of arrival, his position was shelled by units of the Japanese fleet, which paraded through the nearby bay lobbing high-caliber shells into the American position. This performance was repeated nightly. In addition, the small beachhead was subject to repeated raids by Japanese planes. The patient was active in the jungle campaign to enlarge the beachhead. Patient was tired, frightened and hungry most of the time. Sleeping and eating were irregular. He suffered from heat and humidity. Patient's life was repeatedly in jeopardy. At 5 o'clock one morning, a month after the initial landing, while preparing breakfast at his front line position, the patient suddenly became panic-stricken and began to shake all over. He cried constantly. "I felt like running. I did not care where. I just could not stand it where I was." He was removed, in this condition, from his foxhole to a point a few yards behind the forward position where he was seen within 15 minutes by a medical officer. He was then removed to an aid station. "From then on I don't remember anything until I woke up in a hospital the next morning. My head was hurting and my ears ringing." Patient had severe chills. A blood smear revealed the presence of malarial parasites. "Every time an airplane came overhead I would shake all over and my nerves would go to pieces."

Patient was then evacuated by plane to a hospital establishment on another island. After a week he was transferred to another hospital where he was seen for the first time by a psychiatrist—2 weeks after his initial breakdown. Patient was then returned to the United States. After months of treatment and rest in 2 general hospitals, patient was given a 30-day sick leave, following which he was placed on a limited service status. He was then referred to Basic Training Center No. 10, Greensboro, N. C. This patient, together with the 2 others herein reported, were together since their simultaneous arrival in the United States.

One of the patient's outstanding complaints was headache, despite the fact

that no concussion element was present. He complained of extreme nervousness, nightmares, inability to concentrate, extreme irritability, chronic weakness, fatigue and tremor.

*Examination.* On admission, examination revealed a well developed, well proportioned soldier of normal weight and height. Heart and lungs were normal and abdominal examination was negative. Roentgenologic examination and laboratory studies, including numerous examinations for malarial parasites, were negative.

Neurologic examination disclosed moderate tremor of the outstretched hands, and occasionally of the head. Increased perspiration was noted when he talked of his Guadalcanal adventures. Patient objected to having a blood test taken. Failed to cooperate in general ward routine. Generally overintimate with various female employees. During neurologic examination, overreacted markedly to such stimuli as pin-prick and tuning fork. During interviews appeared somewhat indifferent and cautious, answers being given in a somewhat stereotyped fashion. Manner at times was distinctly unsolidary. He attempted to maintain an attitude of reserve but, while talking, agitation became more and more apparent.

When this patient was seen for the first time in the Neuropsychiatric Department his first words were, "Doctor, I am shell shocked." Patient stated that before coming ill he used to take great pride in exhibiting his prowess as a soldier; he talked, with pride, of how he stood up to 30 mile hikes with full field pack. "I could keep up with the best of them. I have a good attitude towards being a good soldier, but my condition won't let me be. I cannot drill with troops because my nerves fire easily, and I am weak all over. I cannot sleep at night. I cannot stand any noise. I am getting no better after all of these months. It's not helping me just being in a hospital." Later, "I could use a 6-months rest at home—not in the army." As to exaggeration of symptoms, "Why should I be a goldbricker? I should like to be classified as more than a goldbricker. A man who has been through what I have been has a right not to be called a goldbricker."

*Case 3.* Age 20, born on father's farm in Nebraska. Parents were in moderate financial circumstances. Rather, born and educated in Nebraska, was a successful farm-owner and operator, dependable and stable. Patient's mother, also Nebraska born, is literate, sociable and well-considered by her neighbors. She is stated to be somewhat nervous and high-strung. There are 11 siblings, all living and well. No nervous or mental disease reported in the family.

*Personal History.* No serious illness or injuries as a child. Home was considered a happy one and patient recalls no chronic family or social difficulties. Began school at the age of 6 and graduated grammar school at 14. No failures. He was well-liked by teachers and playmates. Worked thereafter on his father's farm and occasionally for neighbors. He had many friends both male and female. Normal heterosexual adjustment. No legal difficulties of any kind. Work record is excellent. His National Guard organization was federalized in 1941. He was considered a stable and utterly reliable soldier.

*Present Illness.* Patient landed on the beachhead at Guadalcanal in October, 1942. His experiences were similar in every respect to those of the 2 patients already described. In addition, the patient is known to have had 3 attacks of malaria, about which he states, "I did not worry so much about the airplanes as I did about the malaria. It left me weak and tired." In November, 1942, a month after arrival at the island, a bomb exploded nearby, leaving him somewhat nervous and excited. He was removed temporarily from the front line area, but returned to full duty a few days later. Seven weeks after arrival at Guadalcanal a shell of large caliber landed in his vicinity sufficiently close to partially cover his foxhole with dirt and refuse. He recalls hearing the shell explode, but nothing else. Three hours later he regained consciousness, suffered severe, splitting headache, dizziness, vomiting and nosebleed. He was seen on the spot by a local medical officer. He could not be evacuated immediately and remained at the front all night under shell fire. He was evacuated the

next day and then taken by plane to another hospital establishment. He was then transferred to another island, where he remained about 1 month, complaining of headache, dizziness, nervousness, terrifying dreams, extreme weakness and fits of trembling. This patient was seen by a psychiatrist 8 days after his initial collapse. He was returned to the United States, and treated for many months in two general hospitals. Sent home on a 30-day furlough and then assigned to Basic Training Center.

*Examination.* Physical examination disclosed only tremor and increased perspiration. All laboratory examinations, including smears for malarial parasites, were negative.

In the hospital, patient was generally quiet and cooperative, though given to outbursts of increased activity from time to time. He seemed to prefer to remain alone. Patient was definitely depressed. "I feel weak and I have no energy. I have headache all the time. I cannot stand the heat. I get weak. I cannot sleep. I have nightmares that wake me up. I still jump when I hear noises, especially airplanes. When you get something like that in you it is hard to get it out. They used to bomb us all day long. I cannot take it any more. I cannot get out there on the drill field. I cannot think fast. I cannot do what I used to do." Asked about an easier assignment, "I don't feel that I am good for anything—I honestly don't feel I can be useful. I used to like the army, but since Guadalcanal I lost all of my friends and I don't care about it any more." Later, "It bothers me to be around soldiers all the time. They get me nervous. I used to be able to do anything. I am not a goldbricker or anything like that. I was in the army 2 years and was on sick list only 2 times. I used to enjoy being with soldiers, but since I lost all of my best friends when they got killed I don't any more. I blame it more on malaria fever than anything else. I had it 3 times." Frequently tearful.

*Discussion.* Since World War I, neurosis incurred in war time or under combat conditions has come to be considered pre-conditioned and non-specific. The term "shell shock" for example is supplanted by the less suggestive and less militant "anxiety neurosis"; similarly "gas neurosis" becomes "acute psychoneurotic respiratory syndrome."<sup>20</sup> There has been deemphasis of exciting cause and reemphasis of individual personality and other predisposition factors.<sup>1-3,6-10,13-16,18,19,21,22</sup> It is odd to discover, therefore, in modern milito-psychiatric literature a condition designated by the title "Guadalcanal Neurosis." The use of this term represents a reversion to concepts now unfashionable concerning the origin of the so-called war neuroses. Whether this condition, as described by Smith, represents a new disease appears less important than the circumstances that gave rise to it; the brutal combat situation responsible for this interesting psychiatric aberration, however, redirects attention to the importance of exciting cause. In contrast to the much larger group of soldiers who, because of predisposition, influence of poor family history and psychiatric irregularities, break down early in the course of their military careers,<sup>12</sup> these soldiers broke late, and then only under extreme combat conditions. Among the British during the second Libyan Campaign of 1941,<sup>15</sup> and among the Americans in the North African Campaign,<sup>7</sup> predisposition factors are reported to have been prominent among neuropsychiatric casualties. On the other hand, the majority of acute neuropsychiatric states noted during and immediately following the Dunkirk evacuation occurred in soldiers of normal stability and adjustment,<sup>23</sup> response to treatment was immediate and gratifying.<sup>4</sup> A

somewhat similar situation obtained among bombed civilians of Barcelona and London.<sup>17</sup> In contrast, absence of neuropathic taint and failure to respond satisfactorily to treatment characterize a certain proportion of the Guadalcanal group.

The incidence of a severe and deeply rooted neurosis in individuals not otherwise disposed to mental illness invites attention. This is particularly true in view of prevailing emphasis on the importance of predisposition factors in the psychogenesis of neuroses in war.

**Summary.** 1. Three typical American soldiers of normal emotional and psychologic make up, whose family histories are generally free of neuropathic taint, under the stress of identical campaign and battle conditions have been rendered useless as a front line or support troops. Certain inherent and fundamental patterns of reactivity once regarded as normal, have been so shattered at Guadalcanal that judged solely on the basis of their behavior as soldiers subsequent to injury, these three at present fail to meet the standard of psychiatric competence demanded by army regulations. Their origins are diverse sociologically and geographically. Their behavior was regarded as normal in their respective communities, by their local board physicians, by their induction examiners, and by their superiors in the various stages of training in preparation for combat. Their army records reflect a high degree of adaptability. Only the accident of war and exposure to the exigencies of war exposed their common susceptibilities.

2. Their symptoms are not only similar, but are couched in the same phrases and intoned with the same inflections, with the same air of resignation and defeat. Headache, chronic fatigue and lassitude, tremor, insomnia, nightmares, restlessness, increased tension, hyperacusis, and inability to concentrate are common to all.

3. Behavior, as well as symptoms, is strikingly similar. They have at times been peevish, aggressive, demanding, and occasionally recalcitrant. On these occasions behavior stands in distinct contrast to that noted prior to the Guadalcanal Campaign.

4. All have been exposed to an extreme degree of exhaustion, fear, hunger, thirst, tropical heat and humidity, and jeopardy of life and limb.

5. All were debilitated by malaria before being rendered *hors de combat* by an acute neuropsychiatric disability. One patient suffered an exacerbation of malaria 9 months after leaving Guadalcanal.

6. Two who were rendered unconscious concomitant with shell bursts exhibited no postconcussion or amnesia covering the event, and recalled with great clarity and detail, placement, sight and sound of the exploding shell.

7. Despite the absence of a specific concussion factor in one soldier, amnesia followed the onset of an acute neuropsychiatric condition. This may have been induced in part by the administration of drugs. In addition, this patient, as well as the others, complained of severe headache.

8. All were evacuated through identical medical channels and have been intimately associated for at least 8 months.

9. Neurological examination failed to reveal any evidence of focal neurologic disease with the exception of minor residuals of poliomyelitis in 1 patient. All 3 exhibited hyperhydrosis and tremor.

10. To date, almost 1 year later, symptoms persist with great tenacity despite active therapeutic measures.

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## TUMEFACATION OF SUBCUTANEOUS FAT FOLLOWING THE INJECTION OF INSULIN

A CHEMICAL AND HISTOLOGIC STUDY  
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In spite of the fact that insulin has been in widespread use since 1930, no one completely understands its effect on subcutaneous fat following its injection. The results have been paradoxical because, on



the one hand, marked atrophy may follow and, on the other, fatty tumefaction may result. Alpert and Ferguson<sup>1</sup> (1939), reporting on a 7-year study of diabetics at King's County Hospital, found that 7.4% of their patients developed lipoid atrophy. Joslin and his associates<sup>2</sup> found that atrophy occurred in 18.4% of their series of diabetics.

Fat tumefaction at one time was held to be uncommon. As a matter of fact, Bader and Vero<sup>3</sup> indicated in their report in 1937 that only 1 previous case (Rowe and Garrison)<sup>13</sup> had been described in the American literature. Goldner,<sup>7</sup> in a recent review of the subject, states that only 18 cases had been reported in the world literature. We feel, however, that this phenomenon is not exceptionally rare. Previous attempts at study of these tumefactions appear to have been limited entirely to histologic examinations of biopsy material. We have been unable to find a chemical study of the constituents of these lipoid tumors. Marble and his group<sup>10</sup> conducted detailed analyses of the lipid material in fat atrophy. By the methods employed, no differences between normal and atrophic fat could be detected except a decrease in the amount of neutral fat in the atrophic area. In the case to be described, the opportunity for chemical study of lipomatous material was afforded.\*

**Case Report.** The patient is a 12 year old white female who was first admitted to Charity Hospital when she was 2½ years old with the diagnosis of pneumonia, and was coincidentally found to have diabetes mellitus. She remained in the hospital for 2½ months and was regulated with much difficulty. In the intervening years, the diet was changed very little. Unmodified insulin was consistently given subcutaneously in the deltoid regions of both arms. After 2 or 3 years, swellings at the injection sites became apparent, and these increased in size slowly (Fig. 1).  
**Physical Examination.** The patient is a well proportioned, intelligent white girl, not appearing ill, and definitely retarded in growth. The outstanding findings are bilaterally symmetrical enlargements overlying the deltoid muscles. These are at the sites injected with insulin over the 9½-year period. On palpation, these areas are rather soft, giving the impression of fluid under some slight tension. The borders of the masses blend with the surrounding subcutaneous tissues. The areas measure 8 x 6 x 3 cm. and exhibit none of the characteristics of inflammation.  
**Methods and Material.** Using novocaine anesthesia and under aseptic precautions, a mass of material was removed from the center of the tumefaction overlying the left deltoid. For a normal control, fatty tissue was obtained from the abdominal panniculus of an individual who had met a sudden death. Both specimens were placed in containers filled with solid carbon dioxide and were immediately transported to the laboratory where the following procedures were undertaken.  
 The limited quantity of tissue samples available necessitated the use of the residual materials from the moisture determinations in ascertaining the percentage of total lipids present. The alcohol-ether extract was taken up in ether and filtered to remove traces of extraneous material. This filtrate, dried under vacuum, was divided into 3 portions and used as follows: (a) determina-

\* We are indebted to Dr. K. S. Markey, Chief of Oils, Fat and Protein Division of the Southern Regional Research Laboratory, United States Department of Agriculture, for his assistance and particularly to Mr. B. A. Smith and Mr. F. G. Dollear who performed the quantitative chemical analyses.

tions of free fatty acids, saponification number, saponification equivalent, unsaponifiable material, cholesterol, and total fatty acids; (b) determination of iodine number; (c) determination of phosphorus.

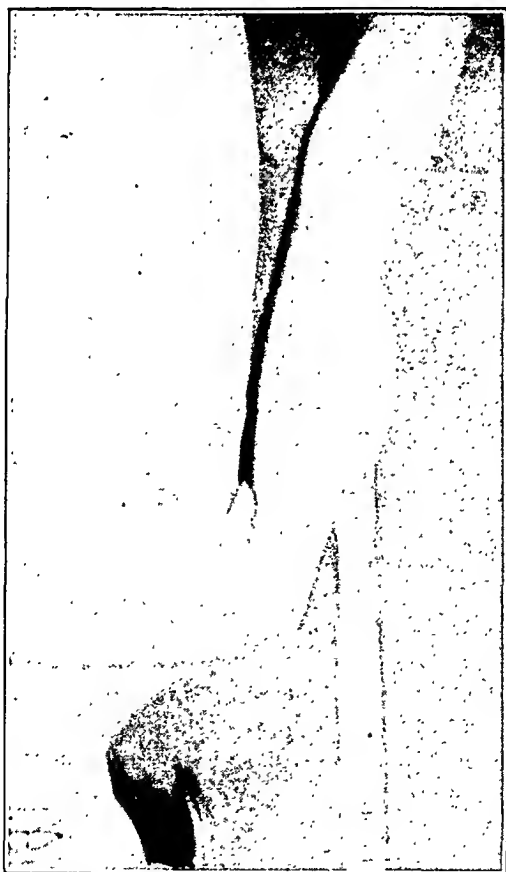


Fig. 1.—Site and size of tumefaction.

**Moisture.** Four-gram samples of tissue were weighed into tared casserolles, minced with scalpels, the scalpels rinsed with alcohol and ether, and the solvent removed on a steam bath. The casserolles were transferred to a vacuum oven and dried to constant weight under a vacuum of 30 mm. at a temperature of 75° to 80° C. About 18 to 24 hours were required.

**Lipids.** The dried residues from the moisture determination were transferred quantitatively to Soxhlet extraction thimbles with absolute alcohol, followed by a small portion of ether. The samples were extracted in Soxhlet apparatus on a hot plate with absolute alcohol for 8 hours. The alcohol was evaporated and the extraction process repeated for a 12-hour period with anhydrous, fat-free ether. The ether was evaporated and the samples dried to constant weight in a vacuum oven at 60° C. under a vacuum of 30 mm.

**Free Fatty Acids.** Two and one-half gram samples of filtered lipids were weighed into saponification flasks, 50 mm. of hot neutral alcohol added, and titrated with freshly standardized 0.1 N alcoholic potassium hydroxide reagent to a pink color with phenolphthalein as indicator. The free fatty acids were calculated as oleic acid.

**Saponification Number (Koettstorfer Number).** These values were determined according to the "Official Tentative Methods of the American Oil Chemists' Society," revised to 1940.<sup>12</sup>

Residues from the free fatty acid determination, corrected for the alkali added in neutralizing the free acids, were used in this determination. Saponification Equivalent. These values were calculated from the data obtained in the saponification number, according to calculations of T. P. Hilditch.<sup>8</sup>

Unsaponifiable Matter. The method used was that recommended by L. V. Cocks.<sup>9</sup> The samples used were the residues from the saponification number determination. The volume of alcohol in the flask was evaporated to approximately 25 mm. before extraction with ether.

Total Fatty Acids. Samples used were the aqueous layers and washings from the determination of the unsaponifiable matter. These solutions were collected in casseroles, the alcohol and water evaporated on a steam bath, and the residual soaps dissolved in water and again dried. The soaps were taken up in water and the fatty acids liberated with 1:3 nitric acid in a warm solution. The two layers were quantitatively transferred to a separatory funnel with water and ether. The fatty acids were then extracted with 3 successive treatments with small portions of ether. The combined ethereal extracts were washed free of mineral acids with water and dried to constant weight in tared flasks, first under a current of dried carbon dioxide, and finally in a vacuum oven.

Iodine Number (Wt%). Iodine numbers were determined according to "Official and Tentative Methods of the American Oil Chemists' Society," revised to 1940.<sup>12</sup>

Sterol (as Cholesterol). These determinations were made according to the method of Windaus.<sup>13</sup> The sterol fraction was calculated as cholesterol.

Phosphorus. Phosphorus determinations were made according to a method reported by H. W. Gerritz<sup>14</sup> and modified by use of a magnesium nitrate alcohol reagent for digestion of the sample.

Phosphatids. These values were calculated from the percentages of phosphorus with a factor reported by H. M. McLean and I. S. McLean.<sup>11</sup>

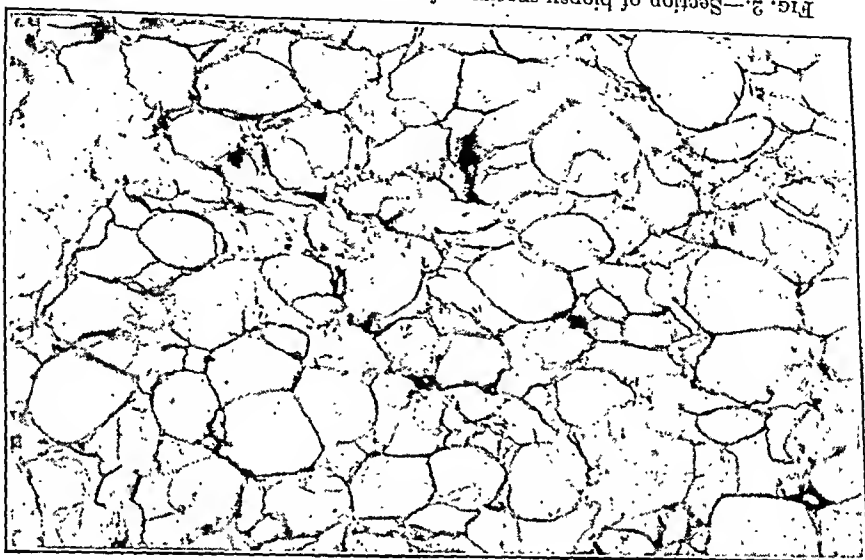


Fig. 2.—Section of biopsy specimen from area of tumefaction. ( $\times 200$ .)

*Histologic Examination.* The sections (Fig. 2) show adipose tissue made up of fat cells of normal size and appearance. A few

\* By Dr. Charles E. Dunlap, Assistant Professor of Pathology at Tulane Medical School.

fibrous trabeculae are present in which blood-vessels and small nerves are seen. No inflammatory changes or other abnormalities are present. In short, this tissue is histologically indistinguishable from normal fat. Inflammatory reactions are inconstant, being reported by some authors and denied by others.

TABLE 1.—COMPARISON OF LIPIDS IN NON-DIABETIC AND DIABETIC TISSUES EXPRESSED AS PER CENT OF TOTAL MASS

	Non-diabetic tissue (%)	Diabetic tissue (%)
Moisture	17.8	23.4
Lipids (alcohol-ether extract)	79.6	70.1
Lipids (moisture-free basis)	95.5	91.4

TABLE 2.—COMPARISON OF LIPIDS IN NON-DIABETIC AND DIABETIC TISSUES WITH PARTICULAR REFERENCE TO THE CHARACTERISTICS OF THE LIPIDS

	Non-diabetic fat	Diabetic fat
Free fatty acids (as % oleic)	0.56	0.51
Saponification number	192.8	195.7
Saponification equivalent	290.9	286.7
Iodine number (Wij's)	69.6	70.3
Unsaponifiable matter (%)	2.88	1.89
Sterols (as cholesterol):		
As % of unsaponifiable matter	12.6	18.6
As % of fat	0.363	0.352
Total fatty acids (%)	92.2	92.7
Phosphorus (%)	0.027	0.023
Phosphatids (calculated from P determination)	0.582	0.683

TABLE 3.—COMPARISON OF LIPIDS IN NON-DIABETIC AND DIABETIC TISSUES SHOWING WATER CONTENT, TOTAL FATTY ACIDS, TOTAL CHOLESTEROL AND PHOSPHOLIPIDS

	Non-diabetic tissue	Diabetic tissue
As received	As received	As received
Moisture-free basis	Moisture-free basis	Moisture-free basis
Water* (%)	17.8	23.4
Total fatty acids (%)	73.4	65.0
Total cholesterol, mg./100 gm.	289	321
Phospholipids, mg./100 gm.	463	624

\* This value is approximate due to method used in handling samples.

Discussion. In Table 1 a comparison has been made between the normal tissue and the diabetic tissue as received in the laboratory. Because a significant difference\* was observed in the amount of moisture in the normal and diabetic tissues, studies were made on the fats extracted by the use of alcohol ether. In Table 2 the data obtained from detailed examination of the constituents of the two fats are presented. In Table 3 total fatty acids, cholesterol and phospholipids are indicated. It will be noted that the total values do not add up to 100%, indicating that the tissues contained other constituents than water and fat.

It will be observed from examination of the data presented that there is very little difference in the non-diabetic and diabetic fats except in the unsaponifiable matter and phosphatids. These values

\* Perhaps due to the greater amount of blood in the diabetic tissue.

are higher in the diabetic fat. Marble and Smith<sup>10</sup> arrive at a similar conclusion from their analyses of non-diabetic and diabetic tissue both in the human and the rat, except in the matter of neutral fat. They feel that the type of fat which disappears in fat atrophy is neutral fat.

An effort was made to present the analytical data in this report in such terms as to afford comparison with the data of Marble and Smith.<sup>10</sup> A review of their findings shows that those values reported as neutral fat were empirical and calculated from data not furnished. As the methods and data reported by Theis<sup>14</sup> represent values apparently differing from those to be found by Marble and Smith<sup>10</sup> calculations of neutral fat were omitted in this report.

It would seem then that the fat tumefaction in our case is composed of essentially the same constituents as non-diabetic fat, the difference being one of degree.

All writers on the subject appear to be in agreement that lipid tumefaction following insulin appears chiefly in children. The explanation of this phenomenon remains obscure. Depisch<sup>4</sup> believed that it is due to repeated traumata at the same site, pointing out that in morphine addicts these changes are not observed because the site of injection is varied. It is of passing interest that one of us (V.J.D.) has not observed local tumefaction, although he has given many pollen injections at the same site over a period of years. Other explanations concern themselves with irritative substances in the insulin such as triacetol. The hypothesis that appeals to us is that of Gellerstedt,<sup>5</sup> who ascribes the phenomenon to a local physiologic assimilatory effect of the insulin, with localized fat retention. In our case, after the site of injection had been changed, there was a measurable reduction in the size of the tumefactions.

**Summary.** A case of localized fat tumefaction following insulin administration is presented. Detailed chemical analyses and histologic study are reported.

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# PROGRESS OF MEDICAL SCIENCE

PEDIATRICS

IRVING J. WOLMAN, M.D.  
UNDER THE CHARGE OF

PASSED ASSISTANT SURGEON (RESERVE), U. S. P. H. S.

## MAJOR MOTILITY PATTERNS OF THE CHILD'S DIGESTIVE TRACT: A REVIEW

BY IRVING J. WOLMAN, M.D.

This Review aims to summarize present-day knowledge of major patterns of digestive tract motility in infancy and childhood. The sections of this report have been arranged according to the normal sequence of passage, commencing with sucking and swallowing and concluding with emptying of the colon. The original plan had been to focus attention exclusively upon the movement of food. But aerophagia and intestinal gas are such prominent features of this age period, especially in the infant, that appreciable space has been given to this subject also. Research studies of recent date have been selected as the bases for exposition whenever available, though older contributions have often been called upon to maintain the continuity of the account.

More comprehensive bibliographies than given here will be found in Reiber<sup>88c</sup> (swallowing); Henderson<sup>44</sup> (Roentgen ray appearance of stomach and intestines in newborn); Soveri<sup>101</sup> (aerophagia); Haas<sup>36</sup> (etiology of pyloric stenosis); Wolman<sup>121</sup> (gastric secretions); Kerley and LeWald<sup>66</sup> (roentgenology of digestive disturbances); Demuth<sup>20</sup> (milk digestion); and especially Freudenberg<sup>28</sup> (the older literature on digestion in childhood); and Alvarez<sup>2</sup> (survey of gastro-enterologic problems in general).

The principal source of information of alimentary movements has been roentgenology. In the following presentation the reader should understand that all descriptions of motility patterns were secured by this procedure, except when other techniques are specifically mentioned.

An analysis of the intricate neurologic mechanisms and chemical secretions which regulate motor activity has not been included. Presentation and evaluation of these complexly interacting influences would obscure the broad contours of normal motor function which this Review aims to portray. Furthermore, not much research has been done with reference to the distinctive functioning of these factors in children.

Descriptions of the almost numberless varieties of major anomalies and their symptom-complexes have also been omitted, except for congenital hypertrophic pyloric stenosis and a few lesser dysfunctions displaying altered gastro-intestinal motility as a dominant feature. McIntosh and Donovan<sup>73</sup> have discussed the derangements and disturbances resulting from anomalies in intestinal rotation.

**Deglutition.** Ingestion of food is the primary purpose of swallowing in man; but as Negus<sup>77</sup> mentions, swallowing also removes the bacteria and secretions draining into the nasopharynx from nose, paranasal sinuses and middle ear. Similarly bacteria carried upwards from the lungs by ciliary action are swallowed, to be destroyed in the stomach. The act of deglutition or swallowing can be subdivided conveniently into three stages:

*Mouth.* In the older child, as in the adult, the mouth actions involved in eating and drinking are more or less under conscious and voluntary control.<sup>77</sup> Food is grasped by the lips, masticated by the fauces by the tongue, teeth and cheeks, and forced back through the fauces by the curved arching tongue. This tongue movement seals off the pharyngeal lumen for the moment.

In the nursing infant, however, and particularly in the newborn infant, the nervous control lies on a lower more involuntary reflex level.<sup>86c</sup> Breast nursing, according to Feiper, begins with the "search" reflex<sup>86c</sup>—the rest-less head and lip movements exhibited by the hungry newborn when warm objects are placed in contact with or close to his face. On finding the nipple the baby presses his face firmly against the maternal breast, and the mother must press back her skin from the baby's nose in order that he may breathe while nursing. The sucking act itself has two phases: First the baby exerts suction by dropping the lower jaw; this draws milk from the depths of the mammary gland into the collecting ducts of the nipple, and may extract a little milk. Then the nipple and the tip of the breast becomes squeezed by mounting pressure as the lower jaw rises upon the upper, while at the same time stroking results from elastic retraction of the nipple and an opposing tongue movement. In this rhythmic coordination of jaw, cheek and tongue muscles the interaction of a number of distinct and different neuromuscular reflexes can be demonstrated.<sup>86c</sup>

In the infant fed from a bottle the movements of sucking are somewhat different. The rubber mouthpiece of the artificial nipple is less supple and yielding than the maternal nipple and usually projects more deeply into the mouth. With a rubber nipple, closure of the infant's jaw squeezes milk into the mouth; when the jaw relaxes the nipple resumes its original shape while air from the oral cavity rushes into the interior of the bottle. Other factors which help to eject milk into the baby's mouth are weight of the fluid column and momentary waves of "recoil" positive pressure as air bubbles rush into the bottle.<sup>40, 54, 87</sup> Generally speaking, the breast-fed baby has to work harder to get nourishment than does the one nursing from the bottle, except when the rubber nipple holes are very small.

*Pharynx.* In this second stage a peristaltic type of movement, involving the larynx along with the rest of the pharyngeal structures, thrusts the food from the palatine pillars to the esophagus. At the same time the esophagus opens and the normally lower intrathoracic pressure exerts a suction effect, particularly during inspiration. In fact, suction may draw food into the funnel-like mouth of the esophagus in advance of the peristaltic wave which descends to propel any lagging bolus.

The infant seems to be able to suck, swallow and breathe uninterruptedly for a long period of time without experiencing choking or other difficulty. The adult, on the other hand, must temporarily suspend respirations with every swallow, while the food flows across the laryngeal opening. This pause takes about 1.5 seconds.<sup>17</sup> The explanation for the difference between infant and adult swallowing, according to the traditional theory first proposed by Hasse,<sup>41</sup> is that the infantile larynx

rises high above the pharyngeal floor, so that fluid flows in lateral channels on either side instead of crossing the orifice. This would be analogous to conditions existing in herbivora and cetaceans who can eat and breathe simultaneously and in whom the elevated larynx reaches almost to the nasopharynx.<sup>77</sup>

When a hungry baby is given a feeding he sucks very rapidly, making 40 to 90 buccal cycles per minute for the first few minutes. Feiper<sup>47, 866</sup> studied the time relationships between sucking, deglutition and respiration in nursing infants by means of direct kymographic tracings and by Roentgen kymography. When the infant nurses steadily on a bottle, there takes place with each respiratory cycle either one sucking and one swallow-ing movement, or two sucking and two swallowing movements, or two sucking and one swallowing movement. The swallowing act takes place either in the instant between inhaling and exhaling, or the instant between exhaling and inhaling. During these split-second pauses the feeding flows over the larynx for from 0.15 to 0.18 second, the rhythm of breathing remaining unbroken. By snapping Roentgen ray films of infants during the moment of swallowing, using 0.05 second exposures, Hofmann and Feiper<sup>47</sup> were able to demonstrate radio-opaque feedings silhouetting the surface of the larynx. These observations fail to support the lateral channel theory of Hasse and denote that the intrapharyngeal mechanisms of swallowing in the infant are quicker but of the same nature as have been established for adults. Feiper<sup>866</sup> points out that such quick acting coordinations between sucking, swallowing and breathing must be regulated by reflex centers in the central nervous system.

*Esophagus.* Food is driven through the esophagus to the stomach by peristalsis. Gravity furnishes but moderate assistance as is shown by the ease with which babies can nurse while in the supine position. The mouth of the esophagus is normally kept tightly closed except when food is being swallowed. Otherwise the stomach would receive a fresh bolus of air many times a minute, one with each inspiration. A specialized band of fibers, the cricopharyngeal sphincter, fulfills the valvular function. Feiper and Isbert<sup>88</sup> studied esophageal movements in infants by means of small rubber balloons placed *in situ*. During sleep or resting there were no peristaltic or tonic changes. In the waking state, under the conditions of the study, swallowing movements could be initiated by sucking on dry nipple.

Aime and Lelong<sup>1</sup> studied in detail the appearance and behavior of the esophagus in 100 normal infants under 1½ years of age. For the greater part of the interval between meals the esophagus was collapsed and empty. During deglutition the esophagus was found to have a lumen as wide as the shadow of the vertebral column. The shape was inconstant and changeable with slight curves and partial constrictions, ballooning out above the diaphragm when food was present. Swallowing liquids was always accompanied by an abundance of large air bubbles standing out clearly in the Roentgen ray films. With a thick meal of gruel or puree, in contrast, the quantity of air ingested was much less and the bubbles were small and sparse. Infants fed at the breast ingested as much air as did those fed on the bottle. After a meal, when the baby was upright, the esophagus often contained one or several sausage-shaped bubbles of air. In the horizontal position it was more likely to contain liquid material. The transdiaphragmatic segment measured about 1 cm. in length. When open it was tubular; when closed, invisible. To the authors it seemed



that the sphincter action here was accomplished by some external agency,

Before the onset of clinical regurgitation waves of liquid would be seen to rise and fall within the esophagus, increasing progressively in height attained. Ultimately all the esophagus would fill and the upper sphincter open up to permit rejection of a little liquid by mouth. For every completed spitting-out there were many incomplete regurgitative movements. During regurgitation the esophagus was passive and ribbon-shaped, without constrictions, responding passively to the pressure from the stomach. In the course of some 500 routine Roentgen chest examinations on newborn infants, Solis-Cohen and Bruck<sup>100</sup> frequently came across gaseous distention of the esophagus. These examinations were made about 1½ hours after feeding time, when many of the infants no longer had a large bubble of air within the stomach. The authors theorized that this esophageal gaseous distention, which is a highly unusual finding beyond the neonatal period, was evidence of poor functioning of the cardio-esophageal sphincter.

*Cardiaspasm.* The controlling mechanism at the esophageal-gastric junction offers no obstruction to the passage of food or fluid down the esophagus to the stomach, but does prevent loss from regurgitation in the reverse direction when the stomach is emptying. There is a question<sup>76</sup> as to whether or not a true anatomic sphincter is to be found at the lower end of the esophagus, though Hurst<sup>48</sup> declares one can be readily demonstrated, in stillborn infants, as a ring of thickened musculature with thickening of the overlying mucous membrane. In any event, there is universal agreement that physiologic sphincter activity does occur at this spot, perhaps due to a special group of nerves locally,<sup>57</sup> or to local physical conditions in the form of positive intra-abdominal pressure,<sup>77c</sup> or to external pinching off by the diaphragmatic cruri,<sup>1,50</sup> or to pressure from the liver and distended stomach.<sup>75</sup> Release of this sphincter is necessary for eructation of swallowed air.

Cardiospasm, known also as esophageal achalasia,<sup>48</sup> or preventriculosis,<sup>50</sup> is the term applied to the clinical disturbance which results when the cardiac sphincter chronically fails to open during deglutition. Clinically the disease is characterized by long retention of food in the esophagus, with stagnation, dysphagia, pain in the chest, malnutrition, choking spells and distention of the esophagus demonstrable by Roentgen ray. Treatment consists in stretching the unrelaxed though not hypertrophied sphincter by means of mercury bougies. Though predominantly a disease of adult life, it has been observed in childhood.<sup>6,30,101,118</sup>

A temporary transient cardiospasm may at times occur in the newborn,<sup>8,108,109</sup> producing dysphagia, dilatation of the esophagus, regurgitation of food after a few minutes nursing with no gastric content in the vomitus, and interference with passage of food into the stomach. The spasm of the cardia can be easily overcome by passing a catheter a few times.

*Stomach. Movements.* Alvarez<sup>2</sup> whose contributions to the study of the mechanics of the digestive tract are many and well known has critically analyzed current concepts of gastro-intestinal motor physiology, with the objective of stimulating research on the many problems still in need of elucidation. The comments in the next three paragraphs are from his book.

The motor activities of the stomach are complex, consisting "of all sorts of combinations of waves, systoles, tonus waves, ripples and reversed

waves." The rhythm of contractions is highly inconstant, the combination of descending waves and local contractions being irregular and ever changing. In adult men gross waves called peristalsis proceed from the cardia to the pylorus at the rate of about 3 a minute, ending usually in a systolic contraction of the circular muscle of the pylorus antrum. Following a wave of contraction a wave of relaxation may follow and material may sometimes then leave the pylorus. But probably the most important factor in initiating gastric emptying is a different kind of slow tonic contraction involving the whole muscular wall and increasing the intragastric pressure. This type of contraction appears to be independent of the other waves mentioned above.

Food is held in the body of the stomach for considerable periods without becoming agitated. Kneading and mixing takes place only in the region distal to the incisura angularis, the pars pylorica. It is questionable whether there is a separate sphincter muscle at the pylorus. This so-called "sphincter" behaves as if it were simply the more thickened distal segment of the muscular coat of the pars pylorica. That the pylorus is not of great importance is indicated by the fact that animals<sup>72</sup> and men<sup>96</sup> get along well following its surgical removal.

Gastric emptying takes place probably only when intragastric pressure becomes greater than pressure in the duodenum. A number of local factors influence the relationships between the pressures in stomach and duodenum, the ones most studied being posture, mechanical stimulation from the meal itself, duodenal distention, small intestinal irritations, fat in the bowel, acid or alkali in the duodenum, consistency, chemical status and osmotic pressure of the food, postoperative trauma and excitation of the vagus or visceral nerves. Foods which are liquid or easily liquefied leave early, whereas hard foods, such as uncooked rice, are kept longer. Alvarez<sup>73</sup> comments that "long lists have been made of the length of time required by various foods for gastric digestions, but as yet it is not known what use the dietitian should try to make of this information. . . . The main organ of digestion is the small intestine; attempts to spare the stomach only throw more work on the bowel."

The method most widely used at present for measuring the period required for the stomach to become empty after ingestion of food has been the roentgenology of opaque meals. According to published reports emptying time can be influenced by type<sup>74</sup> and size<sup>110</sup> of the meal, state of hunger,<sup>49</sup> environmental temperatures,<sup>97</sup> psychic influences,<sup>107</sup> exercise,<sup>43</sup> and reflexes from other portions of the gastro-intestinal tract.<sup>84</sup> Such substances and medicaments as enterogastrome,<sup>34</sup> atropine,<sup>46</sup> sulaptyridine,<sup>79</sup> dehydrotachic acid<sup>43</sup> and benzedrine sulfate<sup>76</sup> can inhibit or stimulate emptying of the stomach.

*Shape.* The child's stomach in the resting state, between meals, is contracted and empty except for a few milliliters of secretion and perhaps a small gas bubble.<sup>1,91</sup> A number of studies have been devoted to the roentgenologic appearance when filled with food containing a contrast medium, though Rogatz<sup>91</sup> reported the infant stomach and surrounding landmarks to be easily identifiable without the use of barium or bismuth. The stomach shadow after a meal consists of an upper gaseous portion and a denser lower shadow. A level fluid line usually separates the two portions. Shape, size and position have proven to be highly inconstant and changeable, differing from child to child and with size and consistency of the meal. Barium sulfate is a heavy substance. Its presence seems to cause deep sagging of the greater curvature contour.

Theile<sup>106</sup> thought the various appearances in infancy could be grouped into two main categories: (1) the "bagpipe" or pear-shaped form, with a large circular expanded fundus and a smaller narrow pylorus directed to the right; and (2) the "tobacco pouch" or retort shaped form, with an expanded upper portion and a narrow lower portion, often with the pylorus visible as a projection to the right.

Rogatz,<sup>91</sup> in addition to corroborating these two types of shadows, speaks also of the stomach contracting to a small circular or oval shape when a thick meal is fed, barely reaching the midline, never extending beyond, and with a very small air bubble or often none at all. This third contracted type is accounted for in terms of a "peristolic" function of the stomach. According to Rogatz, as soon as food enters the stomach the walls are stimulated to surround and grasp the food content (peristoleis). With a fluid meal the peristolic effort if excessive may result in vomiting of the gastric content. With a thick semisolid food, which is viscous and less mobile, vomiting is less likely to occur. The tonsus of the gastric wall is greater with semisolid foods because less air is swallowed with thick foods than with liquids and consequently distention of the wall is less.

Wright<sup>122</sup> studied an older group, consisting of 250 normal healthy children from 6 to 15 years of age. The size and shape of the stomach varied widely at all ages, showing especially striking variation as puberty was approached. A "sink-drain" appearance was more common in younger children, a "sharp hook" in older children. In 42% the stomach lay above the crests of the ilium, in 42% below the crests and in 16% at the crests.

For the newborn, Henderson<sup>14</sup> reported that the shape assumed by the stomach immediately following a meal was inconstant from baby to baby, and presented different contours depending upon whether the films were taken prone, supine, oblique or erect. Shape and size were contingent also upon the amount of air present; if infants cried lustily during feeding, air in unusually large quantities would be swallowed. Feeding usually stimulated gastric contractions, as did change of position and massage of the abdomen over the stomach. As a rule, spontaneous contractions were seen only in the pyloric antrum, but were detected occasionally in the middle and upper thirds. Gastric emptying usually bore no relation to visible peristalsis. Deep contractions might cause no evacuation into the duodenum whereas sometimes when no definite waves were visualized considerable amounts of barium would leave the stomach. Recognizable spasm of the pylorus was seen but rarely.

*Gastric Emptying; Factor of Variability.* That the stomach and intestines are subject to lack of regularity in their responses is common knowledge, but the extent of this variability in childhood has not been exhaustively worked out.

Todd and Kuenzel<sup>107</sup> found that for accuracy in adult investigations on emptying of the stomach one must use only trained individuals under adequately controlled conditions. Subjects must be willing, cooperative and familiar with the procedures. Otherwise confusing and inexplicable variations in response will be encountered. Mental disquiet or emotional stimuli are potent factors in causing pronounced alterations in the gastric responses.

Van Liere and Northup<sup>111</sup> have recently reemphasized that test meals taken for study of normal gastric emptying have not been uniform in character or quantity. These authors suggested the general adoption of some one standard meal such as cooked farina 15 gm. in 350 cc. of water

boiled down to a volume of 200 cc. and then mixed with 50 gm. of barium sulfate. When they gave the above meal to 69 healthy male subjects aged 20 to 30 years under standardized conditions of relaxation after overnight fasting, variations were found ranging from 1.5 to 3.3 hours with a mean of 2.13 hours. Although on repeated testing many subjects exhibited remarkable constancy in gastric emptying time, others showed extensive fluctuation. One apparently normal individual had a range from 1.75 to 3.5 hours in tests repeated at weekly intervals. Hence criteria established for gastric emptying must be expressed as a zone of expected fluctuation rather than in rigid absolute figures.

The normal variability of the gastro-intestinal responses during childhood was examined by Macy, Reynolds, Souders and Olson.<sup>65</sup> The subjects of their study were 7 normal boys and girls aged 74 to 117 months who for months had been subjected to the same standardized conditions of adequate diet and living routine. They were thoroughly familiarized with the procedure of being roentgenologically studied, and remained emotionally calm during examination. Under controlled circumstances, about 1 week apart, test meals consisting of 2 ounces of barium sulfate mixed with 4 ounces of water or of milk were given; 18 months later the milk barium series was repeated. The results can be tabulated as follows (in hours):

Gastric emptying time				Emptying time of jejunum			
Range		Mean		Range		Mean	
(hr.)		(hr.)		(hr.)		(hr.)	
1 0-2.8	1 9	1 5-4.5	2 5-4.0	1 3-3.0	2.4	2 0-4.5	3.4
3 6	3.1	3 6		1 0-4.0	3.2		

Similar wide variations were observed in the rate of progress of the meals through the remainder of the intestinal tract, though variations among the different individuals were not as great as the differences between the water and milk meals. Note the great dissimilarity of the responses to the milk-barium meal after the 18 month interval. It is interesting that there were no consistent differences in the ultimate emptying of the colon as shown in roentgenograms made 24, 48 and 72 hours after the ingestion of the meals. No relationships to age were noted. The authors unfortunately did not perform repeat studies on the same child utilizing the same test meal in a shorter more comparable period than 18 months. The results of their tests indicate that the rate and pattern of normal gastro-intestinal motility as revealed by barium depend not only upon the nature of the test meal, but differs appreciably from child to child. To the Reviewer it seems not unlikely, on the basis of a few personal observations made with the stomach tube, that the range of normal variation for any one individual from day to day may perhaps be as great as the range Macy *et al.* encountered from subject to subject. No large group of children has been studied from the standpoint of extent of individual variation, in a fashion similar to that of Van Liere and Northrup<sup>66</sup> with young adults.

In preparing the present discussion on gastric emptying it was found impossible to compare the findings of most investigations with each other. There were great differences in the character and size of the test meals and in the age, sex and constitution of the subjects themselves. Most studies seem to have paid little or no attention to elimination of psychologic effects, in spite of the disturbing element this factor represents.<sup>107</sup> Again the criteria taken to mark the moment of emptying

have been far from uniform. Some workers recorded the time when most of the food has been passed, though some radio-opaque particles still clung to the mucous membranes; others waited until the stomach had emptied itself completely. In most instances the observations were spaced too far apart— $\frac{1}{2}$  to 1 hour intervals—giving the data a great margin of error. Most series of cases were small in magnitude and lacked duplicate determinations for each subject for each test meal as the measure of experimental error.

Furthermore, the results of most studies on comparative emptying times have not been analyzed according to rigid statistical methods. In many experiments comparing one type of meal with another the variations among individual subjects receiving the test meal were of greater magnitude than the difference between mean values for experimental and control emptying times; in such circumstances the number of subjects used must be of appreciable size for any valid conclusions to be drawn. Thus, for example, Hadary, Sommer and Gonce<sup>37</sup> have questioned the conclusions drawn by as careful a group of experimenters as Reynolds, Macy and Souders<sup>30</sup> on the gastro-intestinal Roentgen ray responses of 7 children to test meals of pasteurized, evaporated and base exchange milks. They subjected the data of Reynolds *et al.* to statistical evaluation by the analysis of variance and concluded that the differences in motility times reported for these three milks had not been proven.

Because of the many differences in the test procedures, and since in most reports the number of cases has been small and not well controlled, results of but a few of the more thorough and representative investigations have been summarized in the paragraphs which follow. These show that the trend in the gastric evacuation pattern as the child grows toward puberty is in the direction of gradually extending the gastric evacuation time, save in the first few weeks of life, when complete emptying is sluggish and surprisingly prolonged. In all instances barium was incorporated into the test meal as the contrast substance.

In the newborn Henderson<sup>38</sup> observed that rapid emptying would take place while meals were being taken, to be followed by an interval of diminished motility for an hour or more and then peristalsis and evacuation. As a rule, most of the meal had moved on by  $1\frac{1}{2}$  to 2 hours. Eighty of 110 infants displayed gastric barium shadows after 8 hours and 30 of these still had some remaining at 24 hours. Similarly Findlay<sup>24</sup> studied 12 normal infants under 2 months of age with a milk meal as controls for a pyloric stenosis series. Barium was frequently visible in the duodenum in the film taken immediately after feeding. Nearly all retained large quantities of barium at the 2 hour examination; traces were often visible on the stomach wall after 6 hours.

Kriger<sup>38</sup> noted that for 11 breast-fed infants the emptying time was  $2\frac{1}{4}$  to  $3\frac{1}{2}$  hours after feeding, whereas for 14 artificially fed infants the range was  $2\frac{1}{2}$  to  $5\frac{1}{2}$  hours with a mean of  $3\frac{1}{4}$  hours. Theille<sup>106</sup> reported on a group of 11 breast-fed babies given successive meals of breast milk and of a half-strength cow's milk mixture. The emptying time for the breast milk was 2 to 3 hours, for the cow's milk mixture 3 to 4 hours. He noted that feedings small in volume remained in the stomach as long as regular sized feedings. Frollo<sup>30</sup> studied 8 infants  $1\frac{1}{2}$  to 5 months of age with a milk meal. He reported beginning emptying within a few minutes and complete emptying by  $3\frac{1}{4}$  hours.

Bouslog, Cunningham, Hanner, Walton and Waltz<sup>11</sup> reported on 133

infants aged 1 week to 6 months receiving a routine milk feeding mixture with baryum. Films taken immediately after completion of the meal showed the presence of food in the small intestine in 244 out of 281 examinations. The emptying times for their series of 133 infants after the completion of the baryum meal is shown in the following table:

No.	Empty at 1½ hours	Emptying time from 1½ to 3 hours	Emptying time 3 to 5 hours	Emptying time 5 to 8 hours	Emptying time more than 8 hours	Total number of examinations
2	5	.	.	.	.	281
9	27	.	.	.	.	100
19	56	.	.	.	.	43
27	80	.	.	.	.	
43	123	.	.	.	.	

Buchheim,<sup>12</sup> with 23 subjects 1.5 to 14 years of age, found that appetite began to leave the stomach promptly after ingestion, and that in 1 to 3 hours complete emptying had been achieved. Hainiss and Surányi<sup>18</sup> noted with 40 children, aged 3 to 10, having low blood pressure (systolic 88 or lower) that 35 showed delayed evacuation of the stomach, evidenced by the stomach contours appearing one-half filled 3 hours after ingestion of a barium meal. The arterial hypotonia and slow gastric motility were interpreted as symptoms of disturbance in function of the sympathetic nervous system.

McLay, Reynolds, Souders and Olson<sup>55</sup> studied 9 children, aged 4½ to 8 years, who for months had been subjected to the same standardized conditions of adequate diet, healthful routine and favorable environment. With a series of test meals fed at weekly intervals the following data were obtained:

obtained:

Mean (hr.)	Range of variation (hr.)	
1.9	1.0-2.8	Water
3.1	1.5-4.5	Milk
5.0	3.0-7.0	Meat
4.8	2.5-6.5	Cream
3.3	3.0-4.0	25% corn syrup solution

The slow emptying time of the carbohydrate meal was ascribed to the high osmotic pressure, a factor which is known to slow gastric emptying. Carmine, 0.2 to 0.3 gm., was given to 7 of these children in another series of tests 3 weeks apart.<sup>66</sup> The average emptying time of the stomach was 257 minutes with the test meal alone; with the test meal preceded by the carmine the average was 177 minutes. However, the later progress of the carmine-containing meal was slower, so that the emptying times for the digestive tract as a whole were about the same for the carmine-barium meal as for the control.

Hadary, Sommer and Gonce<sup>27</sup> recorded gastric emptying times for 7 children between the ages of 4 and 13 given 5 different milk drinks—pasteurized milk, homogenized milk, chocolate milk, base exchange milk and reconstituted evaporated milk. On statistical analysis of the data no significant differences in evacuation time were noted among these milk. By 2 hours the stomach shadows had become reduced from 80 to 25% in scattered fashion, by 4 hours a good number were empty, and by 5 and 6 hours only a very few still contained residue. *Congenital Hypertrophic Pyloric Stenosis*. The gastric motor disturbances consist of projectile vomiting, delayed emptying with prolonged retention of food, great peristaltic waves visible through the abdominal wall, abdominal cramps and constipation. The chief anatomic features

are muscular thickening of the pylorus and hypertrophy and dilatation of the entire stomach itself. The cause is as much a mystery as when the condition was described in 1777 by George Armstrong.<sup>25</sup> Of the hypotheses proposed in explanation, the more important seem to be: (a) the anatomic, which holds that the pyloric sphincter hypertrophies because of the relative obstruction caused by a malformed elongated canal<sup>19</sup> or from anomalous attachments of the pylorus;<sup>21</sup> (b) the neurogenic, which postulates an overstimulation or imbalance of the vagal sympathetic equilibrium as the incitement to pyloric hypertrophy and spasm;<sup>3</sup> and (c) the constitutional or hereditary hypothesis which postulates genetic bases in view of the high frequency shown by siblings<sup>22</sup> and the greater tendency for uniovular twins than fraternal twins to be affected.<sup>27</sup> There is no explanation why the condition is more common among first-borns (52% of Robertson's<sup>28</sup> 278 cases were first-borns, whereas but 41% of the infants born in Toronto in that year were first-borns), nor why four-fifths of all patients are males.

The pyloric disturbance must have its origin *in utero*, for well-developed tumors are often encountered clinically and at operation in the first 10 days of life and in prematures. The lesion has even been found in a 7 month fetus, as described by Cautley and Bent.<sup>14</sup> Hence it seems proper to regard pyloric hypertrophy as a *congenital* lesion, with associated spasm developing in the 2nd to 5th week to produce the vomiting and allied symptoms. The presence of the tumor itself is not the sole factor in the production of disturbances, as shown by the sporadic cases in which little or no vomiting occurs,<sup>7,74b</sup> the scores of patients who have been relieved by thick feedings and phenobarbital, atropine or eumydrin,<sup>53,112,113</sup> and the persistence of characteristic radiologic changes for some years after all clinical phenomena have subsided under non-surgical treatment.<sup>95</sup>

Roentgenography has been repeatedly resorted to in the study of suspected cases of pyloric stenosis. The conclusions of different observers are not in agreement with regard to delay in emptying time. Thus, Findlay<sup>24</sup> compared by Roentgen ray 12 normal infants having hypertrophic pyloric stenosis with 12 normal infants as controls. In hypertrophic pyloric stenosis the onset of passage of the meal into the small intestine was delayed usually, sometimes from 30 to 60 minutes, yet in some undoubted examples later verified by operation the opaque meal entered the duodenum at the same rate as in the normal child. Final emptying was prolonged as a rule. Thus no sharp line of distinction could be drawn between the infants with pyloric stenosis and the normals. In the patients treated by pylorotomy, roentgenograms after 4 weeks showed motility and emptying back to normal. In those treated medically with eumydrin, however, as long as 6 to 8 weeks after all symptoms had disappeared positive Roentgen ray findings when originally present were still persistent. On the other hand, Calvin and Deneholz<sup>13</sup> reported that with a test feeding of 2 ounces of milk containing 2 tablespoons of barium sulfate, hypertrophic pyloric stenosis could almost always be differentiated from pylorospasm by the relative amounts of barium in the stomach and small bowel after 4 hours. In 51 of 55 cases having stenosis, established by later operation, the stomach showed residual retention at 4 hours of 60 to 100% of the barium meal, as estimated from the shadow size. In 23 other cases having pylorospasm as shown by subsequent clinical course only 2 had more than 25% retention at 4 hours. Meuwissen and Slooff<sup>74</sup> described visualization of the pyloric canal when the child is Roentgen rayed lying on the right side following a barium meal.

In normal children the length of the canal was but 1 to 2 mm. In 7 cases with severe clinical symptoms and treated by operation the length was 16.5 to 24 mm. In most of these the stomach showed delayed evacuation, though several were one-half to three-fourths empty before 4 hours were up. The contrast shadow of the pyloric antrum stopped abruptly at the pyloric canal and had a clear linear contour. In 7 other cases the canal was shorter, 4 to 11 mm. Only 2 of these were operated on; the others recovered from the vomiting and other symptoms with non-surgical treatment. It is interesting that in some patients the pyloric canal at times appeared fine and narrow, at other times wide and filled. These changes were ascribed to contraction and relaxation of the pyloric muscle. The authors concluded that intermediate borderline cases are not uncommon and that when the pylorus length is more than the critical length of 6 or 7 mm. the clinical picture of pyloric stenosis becomes strongly evident. In the opinion of Jochims<sup>51</sup> and Runström and Wallgren<sup>95</sup> this finding of an elongated pyloric canal is a much more reliable diagnostic sign of pyloric stenosis than are prominent peristaltic waves, delayed emptying time or enlargement of the stomach. Runström and Wallgren<sup>95</sup> reexamined within a year 8 of 22 patients having the clinical features and the above diagnostic Roentgen signs. The appearances on the whole were unaltered, though the pyloric canal was perhaps broader at times and longitudinal folds more prominent. The stomach was still hypertrophied and gastric waves could be seen. Of 96 other children age 1 to 9 years who had once been diagnosed as hypertrophic pyloric stenosis and were now symptom-free, all but 4 still had an abnormally long pyloric canal, though patulous. The stomach does not return to normal functioning instantly after operation. Faber and Davis<sup>23</sup> followed roentgenographically 10 pyloric stenosis babies. On receiving 1 teaspoonful of barium sulfate in 1 ounce of water within 1 to 5 hours after the Fredet-Rammstedt operation, complete gastric retention lasting from 3 to 11½ hours was noted in all. Emptying began 5 to 18 hours after operation and was not completed until about 20 hours postoperatively. In only 1 infant was the test repeated after the first postoperative day. This infant had slow emptying on the 3rd day but by the 20th day was quite normal. Bau<sup>4</sup> Roentgen rayed 76 children 8 years after operation for pyloric stenosis. In no case did the stomach present any pathologic condition attributable to the operation. The general conclusion to be drawn from perusal of the many reports is that the Roentgen ray offers real aid in diagnosis. However, like most other laboratory tools, there is a significant margin of error. Findings must be evaluated clinically in the light of the other evidence at hand in the individual case.

*Pylorospasm.* Pylorospasm is a diagnosis applied rather freely and uncritically to the recurrent projectile vomiting shown by infants in the first few months of life. The forceful vomiting has been believed to be due to a dysfunctioning tautness of the pyloric musculature. Exit of stomach contents into the duodenum consequently is hampered. Undernourished children may display prominent peristaltic waves visible through the abdominal wall. The differential diagnosis between simple pylorospasm and hypertrophic pyloric stenosis has been summarized by Parmelee,<sup>82</sup> who disagrees with the older opinion, still sustained by Haas,<sup>36</sup> that many cases diagnosed as pylorospasm are essentially mild forms of congenital hypertrophic pyloric stenosis. Alenwissen and Sloof's<sup>74</sup> observations corroborate the latter view. The factors responsible for the symptoms seem to be varied and multiple.



Unquestionably many cases loosely diagnosed pylorospasm owe their symptoms to excessive aerophagy, or to allergy, or to constitutional hyperexcitability, or to gastric irritation from faulty prepared feedings. Modern physiologic concepts of the gastric emptying mechanism are very different from the former orthodox conception of the pyloric sphincter as a "gate keeper." Employing new investigative methods on dogs, Quigley<sup>88</sup> and others<sup>105,117</sup> have obtained evidence indicating that the entire pyloric sphincter region normally executes rhythmic contractions and tends to behave as a unit. The sphincter seems to act in a manner similar to, not contrary to, the action of the antrum and bulb. Normal gastric evacuation is dependent on a pressure gradient from stomach to duodenum adequate to overcome the evacuation resistance. The pyloric sphincter lies open much of the time, even when the stomach is filled with food. During the act of emptying the resistance offered by the sphincter while contracting yields before the greater pressure developed by the pyloric antrum. The presence of foodstuffs or hydrochloric acid in the upper intestine retards gastric evacuation by depressing gastric motility. This retardation occurs in spite of a simultaneous depression of the sphincter and bulb. Emotional states and noxious stimuli delay evacuation by suppressing gastric motility and not by producing pylorospasm.<sup>9</sup> No recent survey has been found applying these new ideas to study of the pathogenesis of the recurrent vomiting in the infantile pylorospasm syndrome.

*Aerophagia; Air in the Stomach.* During infancy and early childhood gas in large amounts is present within the entire gastro-intestinal tract, from stomach to rectum. The chief source of this gas is neither fermentation of food nor diffusion from the blood stream, but swallowing of air. Proof lies in the chemical composition of recovered specimens, which have about the same nitrogen content as the atmosphere,<sup>85</sup> and in the demonstration that patients or animals having esophageal obstruction and fed through gastrostomy openings exhibit no gas in the alimentary canal.<sup>115</sup> Calculation from Charles' law brings out that room air at 21° C. will increase about 6% in volume on being warmed to internal body temperature—an expansion hardly of clinical importance.

Thiele<sup>106</sup> first demonstrated that air reaches the stomach and small bowel in the first 30 minutes of life. Later Bouslog<sup>11</sup> by roentgenography at the delivery table detected air entering stomachs of newborns with the very first inspiration. Paine and Nessa<sup>81</sup> found air in the stomach or duodenum within the first 10 minutes in 5 out of 7 newborn infants; air reached the sigmoid or rectum in all by 4 to 6 hours after birth.

Aime and Lelong<sup>1</sup> and Soveri<sup>101</sup> as well as many older authors have carried out extensive studies on the entry of air into the gastro-intestinal tract of babies. In the period between meals the infant's stomach contains a small quantity of gas; after a fast, none at all. When a feeding is given by stomach tube no air bubble is formed. Gas does not become evident in the stomach when the infant nurses on the thumb or an empty bottle, because no swallowing takes place, except of saliva to a negligible degree. Air reaches the stomach only during swallowing. There is no retrograde current of air during the process of swallowing. Adults differ from infants in being able to swallow air very readily, independently of eating, as demonstrated by Alagnusson.<sup>69</sup>

After a feeding the baby's stomach usually contains a great gastric bubble ("magenblase") which disappears on eructation.<sup>25</sup> The reason

why infants fed on milk swallow more air than older children seems to be that liquids from a bottle reaches the throat in droplet quantity whereas solid foods form large bolus which more completely fill the pharynx during deglutition. Since the stimulus for reflex contraction of the swallowing muscles is the impact of food particles on the posterior pharyngeal wall, bubbles of air from the pharynx are pushed down the esophagus with each swallow of milk.<sup>1,77c</sup> The shape of the nipple plays no rôle, since the source of the swallowed air is the nasopharynx and not the mouth. The position of the child during nursing, whether lying, erect, prone or supine, bears no influence on the size of the gas bubble which forms. Frollo<sup>30</sup> was so impressed by the quantity of air which can accumulate in the first few minutes of feeding that he postulated a muscular relaxation and drop in peristaltic tonus taking place in the stomach wall, stimulated by reflex impulses initiated by swallowing. According to Thelie<sup>106</sup> the shape and size of the stomach after a meal depends largely upon the amount of gas present.

There is disagreement in the literature as to whether the gastric gas bubbles are larger with bottle-fed babies. The differences observed would seem to be resolvable in terms of the size of the holes in the artificial nipples and the time spent in nursing from bottle or breast. Since each swallow of milk carries with it a bolus of air, the longer and more energetically the baby sucks the greater the gas bubble. If the nipple holes are so small that milk cannot flow freely from the bottle, air swallowing becomes prolonged and pronounced. Vlip<sup>123</sup> had noted, by weighing breast-fed infants continually during nursing, that more than 80% of the milk was taken during the first 2 minutes. By 10 minutes more than 95% of the amount secured in 20 minutes would be taken. Vlip<sup>123</sup> demonstrated further with some 60 breast- and bottle-fed infants that the stomach gas bubble was much smaller when the feeding time was cut to 2 to 6 minutes in place of the customary 20 minutes. This change eliminated abdominal symptoms arising from mechanical disturbances due to gas, gave rise to no gastro-intestinal complaints and saved much time for the mothers and nurses. Rubber nipples must have large holes 2 mm. in diameter for such rapid feeding.

In the infant the pylorus lies caudal and anterior to the cardia. Hence, when the baby is held prone or semi-erect, the cardia lies at a higher level and the air will rise into it. Smith and Le Wald<sup>88</sup> have published serial roentgenograms which demonstrate the enormous bubbles which collect during feeding, to escape by eructation following postural manipulation. But when in the supine position air in the stomach rises toward the pylorus and is trapped by the gastric fluid. Thus food which passes from the stomach has to force air ahead of it into the duodenum. It is likely that swallowed air keeps leaking intermittently through the pylorus at all times.

Ålagnusson and Engström<sup>68</sup> found that the quantity of gas in the intestine of infants was greater when the children were prevented from belching after meals and placed on their left sides. Aime and Leong<sup>1</sup> observed that it immediately after nursing an infant is placed on the left side, the liquid contents settle to the fundus and the air rises toward the pylorus. In less than  $\frac{1}{2}$  hour the prepyloric bubble evacuates into the duodenum, not explosively but gradually. Conversely if the infant be placed on the right side the liquid accumulates near the pylorus and the gas rises toward the cardia, and the esophagus will open into air or liquid, depending on the relative proportion of each. They advised that infants troubled with

excessive abdominal gas be placed in the left lateral position for the first ½ hour following nursing and then on the right side for the next hour or two. Soveri<sup>10</sup> reported that an infant's empty stomach can evacuate into the intestine 200 to 300 cc. of air within ¾ hour, much of it being passed within 5 minutes. When the stomach contains a feeding and overlying air, the size of the bubble becomes reduced in size at an equal rate with the passage of the liquid content. When the feeding has been entirely evacuated the air bubble will be gone also.

To be distinguished from normal aerophagia, which occurs normally during deglutition, is excessive or hyper-aerophagia which can give rise to vomiting, colic and tympanites. The most common causes for this, according to Magliano and Hansen,<sup>67</sup> are nasal obstruction, even from simple coryza, feeble nursing, harelip, or cleft palate, and slow flow of milk from the nipple. It is recommended that such patients be treated by forcible belching every few minutes, the attendant exerting light manual pressure upon the abdomen. That aerophagy in excess can give rise to vomiting and other symptoms of gastric distress has been emphasized repeatedly, chiefly by foreign writers.<sup>32,60,70,109a</sup>

**Small Intestine.** *Duodenum.* In infancy, as the stomach empties, the first portion of the duodenum becomes filled as a more or less straight horizontal tube. The second and third portions are seldom visualized due to more rapid activity. When seen these resemble the first portion. The triangular Roentgen ray cap so familiar in the adult organ is rarely seen. With duodenal obstruction resulting from anatomic causes such as constriction by the mesenteric pedicle, the dilated duodenum will fill and stay filled with barium up to the obstructive site.<sup>62</sup> In such cases flat abdominal plates, without barium, may show the enlarged segment distended with a sausage-shaped accumulation of gas.

Cohen<sup>18</sup> has pointed out that plain roentgenograms of the gastrointestinal tract of newborn babies having congenital duodenal obstruction will show the column of air cut off at the site of obstruction, with gaseous dilatation of the more proximal portion of the tract. He confirms Donovan's<sup>22</sup> observation that abdominal roentgenograms without a contrast medium of babies having pyloric stenosis will show stomachs which are large, but not nearly as large as the dilated stomach and duodenal segments seen in duodenal atresia or stenosis. This is an important differential point, for there is no dilated duodenum in patients with pyloric stenosis. Furthermore in pyloric stenosis there is free passage of swallowed air through the entire intestinal tract, whereas in duodenal obstruction distal air will be absent or scanty.

*Jejunum and Ileum.* The small intestine performs the principal work of digestion and absorption of food. Two main types of motor activity take place in the small bowel, according to Alvarez.<sup>2</sup> The rhythmic segmentations and the probably closely related swaying and pendular movements knead the intestinal contents, mix them with the digestive juices, and spread them again and again over the absorbing surface of the mucous membrane. The other chief type is the peristaltic rush. A wave of contraction travels part way down the bowel, frequently commencing in the duodenum about the time a gastric wave reaches the pylorus. A separate though related phenomenon is the pushing forward of a column of intestinal contents which is usually though not invariably associated with a peristaltic wave of the muscle wall. In addition there are a number of other minor types of small intestinal movements, such as tons waves, reverse waves, and systolic contraction of the whole bowel which occurs under adverse conditions as when the circulation is shut off.

Alvarez points out further that the bowel is "polarized," in that waves travel much more easily in one direction than another. It is not inert and passive like a hose. When water is injected into the caudal end of the gut it will go only a short distance and then will be pushed back again. There are so many neurologic and muscular mechanisms at work that the mode of progress of solids and liquids through the small bowel can probably never be summed up by any such short phrase as the so-called myenteric reflex or "law of the intestine" of Bayliss and Starling.

In childhood it is not easy to distinguish jejunum from ileum by Roentgen ray appearances. The characteristic prominent jejunal folds when seen are helpful diagnostic aids, but often they fail to become evident. Zwering and Nelson<sup>124</sup> reported on the roentgenologic patterns of the small intestine in infants and children. They studied 77 subjects ranging in age from 3 months to 11 years, all but 1 in good nutritional condition. Wide variations in appearances were observed at all ages following meals of 1 to 2 ounces of a barium-water mixture. Only 5, including a 5 year old boy with nutritional deficiency, had the appearance considered as "adult normal." With these 5 children, of whom the youngest was 11 months old, the barium moved in a regular continuous stream with even powdery flocculation of the markings and no marked variations in caliber except in areas of active peristalsis. On the other hand, 38 of the infants and children exhibited coarsening or obliteration of the mucosal markings, with segmentation and irregular flocculations. This latter so-called "infantile" pattern was very similar to the "deficiency" patterns described by Macle<sup>64</sup> and Golden<sup>33</sup> for adults. The remainder of the group had patterns transitional between the above two types. Zwering and Nelson concluded that there was no specific age at which the "infantile" changed to the "adult" type of pattern, and that the roentgenologic appearance of the small intestine is not at the present time a reliable criterion for the diagnosis of nutritional deficiency states in childhood.

The above findings are to some extent in conflict with the experience of May and McCrory.<sup>71</sup> In celiac disease (30 patients) and fibrosis of the pancreas (2 cases) they encountered slowing of the segmenting movements and dilatation in the small intestine. Low blood sugar curves secured in the oral glucose tolerance test with these patients were ascribed to defective glucose absorption as a result of sluggish gastro-intestinal motility. When the glucose was given by duodenal tube, to avoid delay in emptying of the stomach, and when active segmenting motion was stimulated in the small intestine by mecholy, a normal rise in the blood sugar curve was "invariably" obtained. At the same time the clumping disappeared from the gastro-intestinal roentgenograms.

May, Reynolds *et al.*<sup>65</sup> have reported on a small series of normal healthy children with various test meals. In all instances, major peristaltic waves were visible in the small intestine only when it was somewhat overloaded with a mass of food. Milk evoked more pronounced wave patterns than did water, though rate of onward movement was slower. When the food approached the region of the ileocecal valve, the intestinal movements were much less active and there was considerable overlapping of the shadows. During the roentgenoscopic observations a sudden propulsion of the contents of the last few inches of the ileum into the cecum was frequently seen. With the different meals it was noted that the children with the most rapid gastric emptying had the least rapid intestinal motility and *vice versa*.

From an analysis of serial Roentgen ray films of 133 infants, aged 1 week

to 6 months, Bouslog *et al.*<sup>11</sup> reported the jejunum rarely visualized with barium. When seen, the jejunum did not present the "feathery" appearance of the adult type. Instead, it appeared sometimes as small isolated masses of barium and other times as a thin continuous line. Occasionally the separated globules were fuzzy in contrast to more clearly cut masses in the ileum. In the ileum the globules arranged themselves more or less isolated from each other, or grouped in the lower right or left quadrant. They frequently remained in the ileum for as long as 5 hours. In the newly born, according to Henderson,<sup>44</sup> the jejunum and ileum when first filled appeared as continuous well-filled loops. After 3 to 4 hours they became segmented, and segmentation usually increased as the examination progressed. The head of the meal reached the cecum in 3 to 6 hours after the meal has been taken. The terminal ileum was often obscured by segmentation in the distal small intestine.

How long does it take for food to pass through the small intestine, from pylorus to cecum? It is not easy to extract answers to this question from the published reports. The great difficulty lies not so much in lack of interest—though specific attention has frequently not been paid to this topic—as in interpreting the overlapping mixture of shadows resulting from the intermittent discharge of food contents into the intestinal lumen. Following is a series of short statements summarizing data from the reports discussed elsewhere in this paper.

Macay *et al.*<sup>45</sup> reported on the variations in what was termed "jejunal emptying time" exhibited by 7 normal children aged 6 to 10 years, though without giving their criteria for distinguishing jejunum from ileum. With a water-barium meal the time ranged from 1.3 to 3 hours; with milk and barium, 2 to 4.5 hours. When the same test was repeated 18 months later with the milk meal, the range was from 1 to 4 hours, wide differences in responses to the 2 tests were exhibited by several children.

Buchheim<sup>42</sup> fed applesauce containing barium to 23 children aged 1 to 14 years. The stomach emptying time ranged from 1 to 3 hours, average 2 hours. The cecum began to show in 1 to 2 hours, though with 1 child, aged 4, it was outlined by 20 minutes. As a rule the small intestines were completely empty in 2 to 4 hours, but in a few instances the passage took up to 6 hours. With Zwierling and Nelson's<sup>44</sup> 77 children 3 months to 11 years of age who received 1 to 2 ounces of a barium-water mixture the material reached the terminal ileum or cecum in 1½ to 6 hours after being taken.

With infants under 6 months of age receiving a milk-barium meal Bouslog, Cunningham *et al.*<sup>11</sup> noted that the cecum often showed partial filling 3 hours after feeding. By 5 hours it was usually well filled. In this series of cases the films taken immediately after completion of the barium meal showed some food already in the small intestine in 2½ out of 281 examinations.

In a small bowel study on 50 newborn infants, Henderson<sup>44</sup> found that the time required for a barium sulfate water meal to enter the cecum ranged from 2 to 6 hours. For 31 of these the times were grouped between 3 and 4 hours.

For adults the time for progress through the small intestine is about the same as that for older children, perhaps a little longer. Under normal circumstances with a milk test meal the first masses of barium reach the cecum as early as 2 hours.<sup>107</sup> By 8 hours most of the food will have passed the ileocecal sphincter, though in some individuals with hyperactivity the ileum may have become empty by 4 hours. With 124 sub-

jects given a water-barium feeding David<sup>19</sup> found onset of cecal filling to spread from less than 2 hours to more than 4 hours; ileal emptying ranged from less than 2 hours to beyond 6½ hours.

The reader should remember that barium sulfate when mixed with a test meal accelerates the rate of passage. This effect has been demonstrated to take place both in the small and large intestines. Consequently, caution must be used in drawing conclusions from comparisons in rates of passage of barium-containing meals, inasmuch as the presence of barium renders all observations abnormal.

*Gas in the Small Intestine.* As described earlier some of the air which accumulates in the stomach during eating is eliminated by eructation, and the remainder is passed through into the intestinal tract. Paine and Nessar<sup>21</sup> took roentgenograms of 46 normal children from the neonatal period up to 10 years of age to determine how patterns of air in the small bowel change with growth. Gas was found distributed about equally between the small bowel and the colon up to 18 months of age. From 18 months to 6 years relatively large accumulations were massed in the colon with less and less in the small bowel. After 7 years, as with adults, no gas was visualized in the small bowel. However, the number of children studied who were over 4 years of age was too small for this stated upper age limit to be construed exactly.

It is well known among roentgenologists that normally gas cannot be visualized in the small intestine in adult life. One assumes that the wall of the adult small bowel possesses greater tonicity and when inactive stays in a state of more complete contraction or collapse than does that of the child. When the adult small intestine contains gas accumulations, one must suspect the presence of ileus, obstruction or nutritional deficiency.<sup>20</sup> Soveri<sup>20</sup> forced 200 to 300 cc. of air into the stomach of infants by stomach tube, and followed its passage through the gastro-intestinal tract by Roentgen ray. When the small intestine was empty, air introduced into the stomach passed all the way through in 1 to 2 hours. Deducting ½ hour for evacuation from the stomach, one obtains ¾ to 1½ hours as the transit time for small intestinal passage. When air was introduced into the stomach within 3 hours after a regular meal its transit through the small intestine took place more slowly, though with greater rapidity than for the meal itself. Soveri noted that when babies were handled or moved about spontaneously the passage time was accelerated.

Magnusson<sup>22</sup> had found that air introduced under experimental conditions into the adult small intestine can pass all the way through in 10 minutes. The entire adult gastro-intestinal tract can become distended with air within ½ hour.

Snow<sup>23</sup> investigated the importance of position as a factor in the Roentgen ray appearances of intestinal gas in infancy. He found that large accumulations of gas would clear away if babies were kept in a semi-inclined bassinet or lying prone or on the right side rather than in the more customary supine position. Snow ascribed the tendency of infantile colic to cease at about 3 months of age to the spontaneous rolling about in bed that babies begin to do at that age. Many clinics place children scheduled for intravenous urography upon the abdomen for the preceding 12 to 24 hours, in order to cut to a minimum the obscuring network of intestinal gas shadows.

**Colon.** Food residues are constantly flowing through the ileocecal sphincter into the cecum where mixing of old and new food residues takes place. One of the main functions of the colon is to conserve the water

supply of the body. It is a sluggish organ with slow movements. As Alvarez<sup>2</sup> describes it, much of the progress of its contents is achieved by day "mass movements" carry material from the transverse colon over into the sigmoid region. During the defecation act the ascending colon assumes an almost globular form; then peristaltic contractions push material from cecum and ascending colon into the transverse colon. About the same time material in the descending colon moves into the rectum. The desire to empty the bowel probably comes when fecal material descends in this way.

The colon of the healthy newborn was reported on by Henderson and Briant<sup>3</sup> in an analysis of the findings in 105 infants aged 2 to 8 days. Fluoroscopic and Roentgen ray of barium enemas were made. Two to 2½ ounces of barium mixture completely filled the colon. Irritability of the large bowel following injection of barium was probably not more marked than in the adult. The mucosal contours rarely showed any defect; any found were believed due to adherent meconium or feces. Hastrations were nearly always present, but were more shallow and less numerous than in older infants and adults. Nineteen of the original group of 105 infants returned at ages varying from 3 months to 1 year for follow-up study. Redundancy of the descending and ascending colon and of the splenic and hepatic flexures was less marked in this older group, evidently due to longitudinal body growth.

With infants in the first year, after a milk-barium meal, Bouslog *et al.*<sup>4</sup> observed that the colon was empty in but 9 out of 281 examinations at 8 hours. At 24 hours in 170, or 61%, the colon was found entirely empty, indicating complete evacuation of the contents. Hastrations were seen sometimes, though not prominent. Some of the infants had spasm, involving the entire colon or but a segment. Peristaltic waves apparently moved the contents with ease. Barium meals were often cut into widely separated segments. Reynolds, Macy *et al.*<sup>5</sup> make no comment as to differences in activity between the child's colon and that of adults.

A moment's reflection makes it evident that the great variations in colon emptying time are tied up in large part with defecation habits. When an individual has been accustomed to performing bowel movements on a regular schedule, the hour of habitual evacuation will exert a significant influence upon the length of time required for a given meal to be passed from the colon, depending upon the hour of day the meal was taken. *Air in the Colon.* Gas received in the colon from the small intestine seems to be absorbed by the mucosa or to leak out slowly and soundlessly. Audible passage of flatus occurs frequently in infants, though the quantity passed per day is a negligible fraction of the quantity swallowed. In the newborn under normal circumstances the entire gastro-intestinal tract from stomach to rectum may contain significant accumulations of air within the first 4 hours postpartum.<sup>6</sup>

Vaugnestein and Rice<sup>7</sup> discovered that imperforate anus in the newborn can be distinguished from rectal atresia by taking a flat Roentgen ray film of the abdomen with the infant held in the inverted posture and the anal dimple marked with a radio-opaque rod. The gas bubble will outline the distal loop of bowel at the site of the congenital obstruction. By noting the distance between the bowel and the external marker the surgeon will receive invaluable information as to the length of the defective segment of bowel and be guided as to the type of operation needed. In 1 of their cases the gap was very slight at 24 hours, though at 14 hours

the terminal end of the colon gas bubble had been far removed from the anal dimple. Consequently in doubtful cases or those poorly distended it is wise to wait 1 to 2 days before deciding on the length of the segment of atretic bowel between sigmoid and anus.

*Spasm of the Colon.* Spasm of the large bowel during infancy and childhood is usually accompanied by distressing symptoms among which anorexia, abdominal pain and attacks of nausea are the most prominent.<sup>9</sup> Production or flare-up of symptoms can result from upper respiratory infections,<sup>88</sup> allergy,<sup>29, 94</sup> faulty autonomic regulation,<sup>86</sup> pathologic conditions of the abdomen such as diseased appendix,<sup>87</sup> and toxic irritants<sup>9</sup> or as a sequel of bacillary or amebic dysentery.<sup>9</sup> Constipation is the more usual symptom,<sup>9</sup> though Rubin<sup>94</sup> encountered infants under 2 months of age allergic to cow's milk who exhibited loose diarrheal stools containing mucus and blood. Fries and Zimor<sup>29</sup> were able to corroborate that the colon in allergic patients responds directly to local presence of the allergen. They administered barium enemas containing a small quantity of the known offending allergen to 9 children subject to gastro-intestinal allergic disturbances. In response to these enemas, 5 of the 9 developed colon spasm as evidenced by abdominal cramps and narrowing of all or parts of the colon with prominent haustra and deep sulci. Control allergen-free enemas with these children gave normal roentgenographic contours, without spasm.

*Number of Defecations per Day.* The number of defecations per day is known to be higher with infants than with older children, but the Reviewer is not familiar with any broad survey of the behavior pattern of this activity in normal children covering increases in age. That the newborn has a bowel evacuation after nearly every feeding is common experience, but the figure for most babies soon falls to 2 to 3 movements daily, and older children have movements once or twice daily. However, as was brought out in a Round Table Discussion of the American Academy of Pediatrics<sup>93</sup> a few years ago, the consensus now holds that a bowel movement daily is not essential for health, even in infancy, provided the baby is comfortable and free from obvious symptoms of distress.

For a small group of 9 children aged 5 to 8 years used by Shepherd, Hummel and Macy<sup>96a</sup> in a study on alimentary tract functioning on diets containing added raw apples or banana, the mean laxation rate over a 30 to 55 day preexperimental control period was 1.5 bowel movements per day; during the 20 to 40 day experimental period the mean rate was 1.7 per day.

For a summary of average rates of defecation during infancy, the Reviewer has tabulated (Table I) data collected in the course of two different infant feeding studies performed on identical groups of Philadelphia children in successive years.<sup>119, 120</sup> Note that the grand mean laxation rate falls slowly as age in months increases. Figures for the first 2 weeks of life have not been included because the number of infants studied at this age was too small for statistical validity.

**Total Gastro-intestinal Transit Time.** The period required for foods to pass through the alimentary tract of the infant was studied by Lésné, Binet and Paulin.<sup>61</sup> Carmine in 0.2 gr. quantities, dissolved in water, was fed and the stools observed for the appearance and later fading away of this pigment:

Age	No. of infants	Appearance time	Disappearance time
1 to 3 mos.	35	8 hr. 30 min.	18 hr. 40 min.
3 to 6 mos.	22	8 hr. 40 min.	19 hr. 50 min.
6 mos. to 1 yr.	15	9 hr. 20 min.	20 hr. 40 min.
1 to 2 yr.	12	10 hr.	23 hr.



These figures, representing averages presumably, demonstrate that the rate of passage becomes slower with increasing age, reflecting the growing length of and greater control over the colon. Breast milk passes through a little more quickly, probably because it is lower in residue:

Disappearance in stool (average)	No.	Appearance (average)	Disappearance (average)
Breast-fed infants	30	8 hr. 25 min.	17 hr. 30 min.
Mixed breast and bottle feedings	40	8 hr. 55 min.	19 hr. 30 min.
Artificially fed infants	51	9 hr. 50 min.	20 hr. 50 min.

Variations from 4 to 12 hours were observed in first appearance time for the same breast-fed baby and from 3 to 20 hours for the same artificially fed baby, illustrating the great irregularities and spontaneous fluctuations which are normal physiologic occurrences.

TABLE 1.—LAXATION RATE OF NORMAL INFANTS IN RELATION TO AGE IN MONTHS

No. of Milk infants†	Age in months											
	1‡	2	3	4	5	6	7	8	9	10	11	12
Mean	2.67	2.63	2.41	2.37	2.28	2.27	2.29	2.02	1.97	1.82	1.75	1.72
1*	3.33	3.13	2.62	2.48	2.43	2.40	2.44	2.28	2.45			
2*	2.65	2.60	2.22	2.09	2.21	2.17	2.18	2.03	1.84			
3*	2.70	2.63	2.42	2.20	2.18	2.32	2.16	2.05	2.00			
4*	2.55	2.38	2.16	2.09	2.22	2.16	2.20	2.05	2.00			
5*	2.58	3.11	3.00	2.93	2.50	2.58	2.27	2.04	1.87			
6*	3.00	3.91	2.79	2.89	2.69	2.48	3.13	2.07	1.94			
7*	2.31	2.19	1.85	1.90	1.86	1.88	1.69	1.41	1.59			
8*	2.26	2.35	2.28	2.34	2.16	2.20	2.21	2.08	1.92			
Mean	2.26	2.35	2.28	2.34	2.16	2.20	2.21	2.08	1.92	1.90	1.83	1.54

\* No. 1, A vitamin-fortified iron-containing modified milk mixture containing 7.4% lactose 2.8% butter fat and 65 to 85 units of thiamine; No. 2, same as No. 1, but with 6.6% lactose, 2.4% butter fat and 1.6% corn syrup; No. 3, same as No. 2, but with 8.2% lactose, no corn syrup and 35 to 55 units of thiamine; No. 4, same as No. 3, but with 65 to 85 units of thiamine; No. 5, pasteurized market milk, with 4 to 4.2% butter fat, boiled for 5 minutes in home; No. 6, same as No. 5, but sonic homogenized and fed unboiled; No. 7, same as No. 5, homogenized at low pressure and fed unboiled; No. 8, same as No. 5, homogenized at high pressure (2500 pounds) and fed unboiled. † Not every infant was followed for the full period of observation.

Kahn<sup>22</sup> studied the duration of alimentary tract passage in 30 healthy infants aged up to 16 months in a total of 200 determinations. Normal feedings were given marked with charcoal or carmine. Striking variations in transit time were detected, not related to the age in months. The times ranged from 4 to 20 hours, the mean being about 15 hours. It was found that when the markers were introduced into different meals throughout the day that the 5 a.m. feeding came through in a mean time of 12 hours, the 1 p.m. feeding in a mean time of 19 hours, and the 9 p.m. feeding in a mean time of 15 hours.

When passage of breast milk was compared with that of artificial formula made of cow's milk, the author stated that breast milk passed more rapidly, though calculation of his published data for infants within the age period in which breast milk was given revealed no significant difference in the mean transit times.

When 2 successive meals were marked with carmine and charcoal respectively, some mixing of the pigments would be apparent in the stools. If, however, there was a wait of 12 hours between the marked meals the pigment in the feces was no longer mixed. It is apparent that individual stools do not necessarily bear any specific relation to individual meals. Interestingly in 1 child passage of 1 marked meal took 66 hours; when repeated, but 23½ hours.

Using the Roentgen ray and barium to study meal passage, Kahn<sup>22</sup> found that the longest period for the stomach emptying was 4 to 5 hours, for the small intestine 7 to 8 hours, for the large intestine 20 to 24 hours. Thus

the differences in speed of passage existing between adults and infants lies almost entirely in the greater rate of movements through the colon. The usual time required by the healthy adult is 24 to 48 hours.

With 13 premature babies weighing 1450 to 2870 gm. at the time of the test and ranging from 2 days to 5 months of age, the duration of passage varied from 6 to 19 hours, apart from 1 instance in which the marker took 42 hours to come through. The mean transit time was 13 hours. Thus there was less variation than with full-term infants.

With 23 infants having dyspepsia and frequent mucus-containing stools the total transit time was 6 to 10 hours. Roentgen ray studies showed the passage through the small intestine to be much more rapid than normal. Gastric emptying time was not accelerated, lasting 3 to 4½ hours, but the small intestine was usually empty by 3½ hours later, and the large bowel had evacuated in 2 to 5 hours. When dyspeptic infants had slower transit times the delay was due to more sluggish evacuation of the rectum. In chronic disease, as is well known, intestinal and abdominal wall atony and slower transport of food may develop. This principle is illustrated in Strickler, Fisher and Lowenberg's<sup>103</sup> study of chronic eczema. Twenty-eight children aged 15 months to 11 years having chronic eczema of diverse types and 5 normal controls aged 18 months to 8 years were studied with a barium meal. The first film taken at 6 hours showed barium residue still in the stomach in 12 of the eczema patients, and in but a single control. As for elimination from the rectum, 3 of the controls were rid of the barium by 24 hours, and the other 2 by 48 hours. In the eczema group, however, 15 still had retention at 96 hours, and took up to 6 days longer for elimination; I went 18 days before excreting all the barium. Many of these passed regular bowel movements during the time the opaque meal was stalled in the colon.

**Comparison of Motility in Child and Adult.** Wasson<sup>104</sup> compared in general terms the motility of the gastro-intestinal tract in infant and child with that in the adult. In the infant food is transported as a steady current through stomach, small intestine and colon without any pause save in the stomach which functions as a reservoir. In the adult the stomach and small intestine empty in approximately the same time interval as in the infant and young child. However, a great delay occurs in the colon which may require 24 to 48 hours longer to empty than the infant colon. This slowing of the colon begins soon after infancy; at a few years of age the colon emptying time may approximate that of the adult. As an individual grows up his sphincters become more vigorous in their action, the contractions of stomach and intestine grow stronger, and localized changes and enlargements develop. Among other changes, the jejunal loops lose their irregular globular pattern and assume the snow-flake appearance of the adult. The outline of the colon too becomes much sharper, with more complete filling and development of the haustral markings. Wasson concluded that the slower rate of evacuation of the adult colon is due to more powerful sphincter control and differently coordinated reflexes.

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## GYNECOLOGY AND OBSTETRICS

UNDER THE CHARGE OF

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## ANALGESIA IN OBSTETRICS

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The purpose of this paper is to discuss the drugs which are given during childbirth; to relieve pain, to provide the loss of sensation, or to render the parturient amnesic for the pain. The terms analgesia, anesthesia and

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amnesia are used to describe the preceding effects. These terms are used interchangeably in this paper.

Efforts to relieve pain during labor reach far into the past. Ploss and Bartels<sup>31</sup> state that, with the single exception of the American Indian, all races of the past and present have attempted to relieve pain during child-birth. Confirmation of this statement is afforded by the wide variety of pain-relieving devices used by primitive peoples. An equally wide, but more effective variety is in vogue today.

Modern efforts at analgesia were initiated by Simpson in 1847 with ether which he soon replaced with chloroform. An ideal analgesic agent is being sought constantly, but so far in vain. An ideal analgesic should be universally applicable, inexpensive and easily administered. It should have no harmful effect on the mechanism of labor, the mother, or the infant. The majority of this discussion will be devoted to the two newest analgesics, preceded by brief mention of older and more familiar analgesics.

**Morphine and Related Drugs.** Morphine and its related compounds have excellent analgesic properties. The use of morphine in obstetrics was popularized by von Steinhuechel in 1902. The analgesia and amnesia obtained were satisfactory, but the fetal and maternal complications were so frequent that it was largely discarded. It is still utilized but it is given only in the early stages of labor. In a recent article Mengert<sup>32</sup> concluded that the greatest fetal respiratory depression occurs during the third hour after the administration of morphine and that it should be avoided in premature labors. He feels that, with careful supervision and with adequate resuscitation facilities, it can be used safely. We use morphine sulfate analgesia frequently at the Hospital of the University of Pennsylvania and feel that it is safe when Mengert's warnings are observed. Although heroin<sup>34</sup> and dihydrid<sup>33</sup> produce less fetal depression they are not widely used. Barbituric Acid Derivatives. According to Stander,<sup>35</sup> barbituric acid derivatives are the analgesic drugs most commonly used in this country. They do not relieve pain but make the patient amnesic. Of these derivatives, pentobarbital is used most widely. Irving<sup>18</sup> and associates, reporting upon its effectiveness in 1934, concluded that it was nearly ideal. The restlessness associated with pentobarbital amnesia is so great that special nursing care is necessary. Its use is contraindicated, for the same reason, in complications requiring rest, such as in cardiac and pulmonary disease. Recently it has been found to depress the fetal and maternal prothrombin time.<sup>11</sup> This effect can be counteracted by the antepartum administration of vitamin K.

**Other Well-known Analgesics.** Rectal ether, chloral hydrate and paraldehyde are given less frequently than morphine or the barbiturates in labor. They do not produce amnesia as satisfactorily as do the barbiturates and do not offer any greater safety.

Nitrous oxide, ethylene, ether and chloroform are well-known inhalation analgesic agents and will not be discussed. Two newer analgesic agents, vinylene and cyclopropane are in the process of evaluation. Karp and Richardson<sup>36</sup> have pointed out the many advantages of cyclopropane for obstetrical procedures. It is less irritating to the respiratory tract than most inhalation anesthetics; it provides adequate analgesia and relaxation for the majority of obstetrical operations; it has no deleterious effect on the kidney or liver and, because it is used with a high per cent of oxygen, it does not reduce the oxygen available to the fetus *in utero*. Our own experience with cyclopropane in Caesarean sections confirms these observations. Very little has been written on the use of vinylene in obstetrics.

Hartmann,<sup>16</sup> in a recent report, regarded it as a safe analgesic agent for delivery. It should not be used in the presence of liver disease or if the

The safety of spinal analgesia has been debated since Cosgrove<sup>8</sup> reported its successful use in 1934 for Cesarean section. Many obstetricians feel that it is unsafe. Two recent reports<sup>2,29</sup> indicate that it is a safe and valuable obstetrical analgesic. They stress the necessity of proper technique and careful observation of the contraindications. Most of the complications with spinal are catastrophic, such as uterine atony with hemorrhage, respiratory failure, intraspinal infections and peripheral vascular collapse. On account of the potential dangers associated with spinal analgesia it can be used only where proper facilities are available.

Completeness demands mention of intravenous analgesia, hypnosis and pudendal nerve block. Although some authors<sup>6,37</sup> think that intravenous analgesia is safe, it is not generally believed to be safe enough for use in obstetrics. Kroger and DeLee<sup>22</sup> reported upon the use of hypnosis to produce analgesia during labor and delivery. They confirmed the observations of others who have used it with success. The low risk to the mother and baby warrant further investigation of this method. No comments need be made on the use of pudendal field block. It is a simple, inexpensive and satisfactory method of relieving perineal pain for delivery. DeLee for years favored more extensive use of this method in his comments in the Yearbooks of Obstetrics and Gynecology. He, likewise, encouraged the use of local infiltration for Cesarean section.

The use of Demerol and the introduction of continuous caudal analgesia are the two outstanding new developments in obstetrical analgesia.

**Demerol.** Demerol (ethyl 1-methyl-4-phenylpiperidine-4-carboxylate) is a synthetic drug which can be given orally, intramuscularly, or intravenously. It combines the analgesic properties of morphine and the antispasmodic properties of atropine, although it is unrelated chemically to either of them. The analgesic effect of this substance, determined by a variety of methods, is less than that of morphine, but greater than that of codeine.<sup>3</sup> Its antispasmodic effect on the smooth muscle of the intestinal, uterine and respiratory tracts has been demonstrated in animals.<sup>9,15</sup> This action is thought to be a direct one on the smooth muscle. It has no effect on the uterine contractions of human beings.<sup>40</sup>

Animal experiments reveal that large doses of Demerol produce excitement, ataxia, spasticity, clonic movements, convulsions and death.<sup>15</sup> Chronic administration of Demerol has led to anorexia and weight loss.<sup>9</sup> Therapeutic doses in man produce side reactions in 25% (Schumann<sup>33</sup>) of the patients receiving the drug by mouth and nausea and vomiting in 29% (Gallen and Prescott<sup>13</sup>). The side reactions include dizziness, flushing, dryness of the mouth, sweating and euphoria. Most of these are transitory. A few reports have been made upon the use of Demerol in obstetrics. A carefully analyzed series of 1000 deliveries has been reported by Schumann.<sup>33</sup> Satisfactory amnesia was secured by 90% of his patients when the Demerol was accompanied with scopolamine. This degree of success is slightly lower than that obtained with nembutal-scopolamine sedation in 1934 by Irving at the same clinic.<sup>18</sup> It is important to note that the dosage of scopolamine used by Schumann was in the same range as that employed by Kirschbaum,<sup>21</sup> who obtained satisfactory amnesic results with scopolamine alone. Only 60% of the patients treated by Gallen and Prescott<sup>13</sup> had satisfactory analgesia. Compared to patients sedated with other drugs, the patients receiving Demerol had definitely shorter labors.<sup>14</sup>

Schumann found that the average duration of labor under Demerol and scopolamine in primipara was 2.5 hours shorter in multipara. Gallen and Prescott reported apnea in 10% of the infants and Schumann stated that 15.29% of his infants required resuscitation. The frequency of stillbirths and neonatal deaths was remarkably low in all reports, and no serious maternal complications were noted.

**Sympathetic Nerve Block.** Sympathetic nerve block, which is the basis of caudal analgesia, is accomplished either by paravertebral or epidural injection. The former method was suggested by Shumacker,<sup>34</sup> and has been studied more intensively since then by Jarvis.<sup>19</sup> Although single injections into the epidural space through the sacral hiatus have been used in this country for years, it remained for Hingson and Edwards<sup>17</sup> to develop a technique in which the analgesic solution could be administered intermittently. Alanalan<sup>26</sup> substituted a small nylon catheter for the malleable needle used by the preceding authors. Block and Rochberg<sup>4</sup> later developed a method of administering the solution by continuous drip. An understanding of the nerve supply of the uterus as described by Cleland<sup>7</sup> is essential to the proper performance of sympathetic nerve block analgesia. Briefly he found that the motor fibers of the uterus are derived from the sympathetic fibers arising in the aortic ganglia, reinforced by ones from the solar, renal and genital ganglia. The sensory nerves of the fundus of the uterus run through the sympathetic chain, entering the spinal cord at the eleventh and twelfth dorsal segments. The sensory innervation of the cervix, and upper portion of the vagina, is derived from the sacral plexus. The sensory and motor supply to the lower vagina, pelvic floor and perineum are derived from the lower lumbar and pelvic somatic nerves.

Epidural block by the serial or continuous caudal technique consists of passing a malleable needle or catheter through the sacral hiatus and injecting an analgesic solution into the epidural space. Metycaine and procaine solutions are used most frequently. The amount of solution injected determines the height at which the block will occur. The needle or catheter is left in place during labor and delivery, and sufficient solution is given to maintain skin anesthesia at the level of the tenth dorsal segment. Skin anesthesia at this level blocks the pain fibers to the fundus of the uterus. For detailed explanation of the various techniques, the reader is referred to the original articles by Hingson and Edwards<sup>10</sup> for serial injection with a malleable needle, Alanalan<sup>26</sup> for serial injection with a catheter, Block and Rochberg<sup>4</sup> for the continuous drip technique and Jarvis<sup>19</sup> for the technique of paravertebral block.

Although continuous and serial sympathetic block are relatively new techniques, they have disadvantages as well as advantages. Below is a partial list of the advantages and disadvantages of this method as reported by McCormick.<sup>27</sup>

*Advantages.* 1. Labor is remarkably free of pain.  
2. The patient retains all of her faculties.

3. It is valuable in cardiac and pulmonary complications.

4. In premature labors the relaxation of the pelvic floor combined with the reduction of fetal depression are extremely desirable. (This is considered by McCormick as the most outstanding contribution of caudal anesthesia.)

5. Uterine bleeding in the third stage is lessened.

6. In teaching medical students, it is possible for the student and instructor to do a rectal examination at the same time.

*Disadvantages.* 1. The procedure requires the constant attendance of someone skilled in its use.

2. It is available to only about 60% of women, those delivered in hospitals (Subtract from this number a large percentage for those women delivered in hospitals without the necessary facilities for continuous caudal analgesia, 9% for failure to insert the needle, and an uncertain percentage for patients considered unsuitable). It is contraindicated by placenta previa, premature separation of the placenta, uterine inertia, disproportion, anemia, drug sensitivity, sacral anomalies, and local infections.

4. There is a possibility of peripheral nerve damage, infection and unrecognized spinal tap.

5. Operative interference is increased.

6. The maternal mortality rate is increased. (Judging from McCormick's discussion of this point, this seems to be the most significant disadvantage.)

Since there is considerable difference of opinion in regard to the desirability of using continuous caudal analgesia, some of the results obtained by different authors are summarized below.

# PAIN RELIEF

Reference	No. of patients	Results
1. Hingson and Edwards <sup>17</sup>	33	Complete relief in all
2. Block and Roehberg <sup>4</sup>	39	Complete relief in all
3. Block and Rotstein <sup>5</sup>	61	Successful insertion of needle in 95%; supplementary anesthesia in 7 patients
4. Siever and Mousel <sup>25</sup>	300	Satisfactory, 288; supplementary anesthesia, 12
5. Lyons and Hansen <sup>25</sup>	200	Satisfactory in 195 patients
6. McCormick <sup>27</sup>	100	Complete success, 40%; partial success, 40%; failed, 20%
7. Lull <sup>22</sup>	553	Complete success, 92%; failed insertion, 38; complete success, 98;
8. McEitroy and Donnelly <sup>26</sup>	200	No comment
9. Jarvis <sup>19</sup>	70	No comment

Insertion of the needle into the sacral hiatus sometimes is difficult, if not impossible, even in the hands of experts. Hingson and Edwards report success in only 91% of their cases. The most frequent cause of failure, in our hands, to insert a needle successfully, is obesity which obscures the usual landmarks. Secondly, inexperience leads to more frequent failures. The third most common cause of failure is sacral anomalies, which are said to occur in 10 to 15% of patients.

With the exception of two of the above reports, excellent results were noted. Hingson and Edwards,<sup>17</sup> reporting the results in 10,000 cases from clinics throughout the country, stated that complete relief was obtained in 81%, partial relief in an additional 12% and failure in the remaining 7%. These authors do not state whether this latter figure includes the cases in which it was impossible to insert the needle.

The frequency of operative delivery is increased under serial caudal analgesia on account of the fact that the presenting part does not reach as low a level in the pelvis as usual, since the block inhibits the normal expulsive reflex; and that, in the absence of the usual resistance of the pelvic floor, the normal mechanism of rotation is disturbed.

The application of outlet forceps is a simple procedure and without danger to mother and infant. On the other hand, the applications of forceps high in the pelvis, or requiring rotation of the fetal head are a more serious matter. Therefore, no matter how skilled the obstetrician, the fetal risk will be increased unnecessarily with the use of serial caudal



analgesia, in spite of the marked relaxation of the pelvic floor which facilitates forceps manipulations.

#### MODE OF DELIVERY

Results	No. of patients	Reference
Spontaneous, 10; operative, 23	33	1. Hingson and Edwards <sup>10</sup>
Spontaneous, 10; operative, 29	39	2. Block and Roehberg <sup>1</sup>
"Does not interfere with spontaneous delivery"	61	3. Block and Roehberg <sup>1</sup>
Primiparas—Spontaneous, 30% operative, 70%	300	4. Siever and Mousel <sup>35</sup>
Multiparas—"Multiparas were able to push the baby out rather easily"	200	5. Lyons and Hansen <sup>25</sup>
"Large percentage of operative deliveries"	100	6. McCormick <sup>27</sup>
"Necessitate increased operative delivery"	553	7. Lull <sup>23</sup>
"Increase in operative delivery"		
"More posterior position and particularly transverse arrests"		
"Breech decomposition and internal podalic version cannot be accomplished"	200	8. McElroy and Donnelly <sup>23</sup>
Spontaneous, 50; operative, 112	70	9. Jarvis <sup>19</sup>

#### EFFECT ON THE FETUS

Results	No. of patients	Reference
One stillbirth; all other babies cried immediately	33	1. Hingson and Edwards <sup>10</sup>
One stillbirth; no fetal apnea	39	2. Block and Roehberg <sup>1</sup>
Two stillbirths and 1 neonatal death; no apnea	61	3. Block and Roehberg <sup>1</sup>
"All cried spontaneously; fetal mortality, 1%"	300	4. Siever and Mousel <sup>35</sup>
No comment	200	5. Lyons and Hansen <sup>25</sup>
Two stillbirths and 3 neonatal deaths, 1 of which is attributed to the analgesia	80	6. McCormick <sup>27</sup>
"All cried almost immediately"	553	7. Lull <sup>23</sup>
"All cried almost immediately"	162	8. McElroy and Donnelly <sup>23</sup>
Apnea, 21; stillbirths, 1; neonatal deaths, 6	70	9. Jarvis <sup>19</sup>

#### THIRD STAGE

Results	No. of patients	Reference
No comment	33	1. Hingson and Edwards <sup>10</sup>
Estimated blood loss less	39	2. Block and Roehberg <sup>1</sup>
"Less than average bleeding"	61	3. Block and Roehberg <sup>1</sup>
"Considerably less blood is lost"	300	4. Siever and Mousel <sup>35</sup>
No comment	200	5. Lyons and Hansen <sup>25</sup>
"Less than with other methods"	100	6. McCormick <sup>27</sup>
"Average in the last 500 cases was 111 cc."	500	7. Lull <sup>23</sup>
196 cc.	162	8. McElroy and Donnelly <sup>23</sup>
Measured average (29), 245 cc.; estimated (17), 208 cc.; estimated (10), 225 cc.	56	9. Jarvis <sup>19</sup>

The uterine motility of the third stage of labor is said to be unusually satisfactory under caudal analgesia, and as a consequence, the blood loss

is small. On the other hand, the frequency of incarceration of the placenta is increased. Vaux and Mitchell<sup>18</sup> and Lull<sup>19</sup> have reported a very small blood loss during the third stage under continuous caudal analgesia. An evaluation of the blood loss in the third stage should not be made without due account being taken of the conditions under which the blood loss was measured. We have observed a greater loss than Vaux and Mitchell, and have attributed our higher average loss to the fact that the majority of deliveries were conducted by internes and externes. Almost all observers are agreed, however, that the blood loss is small under caudal analgesia and that the third stage is short and satisfactory in most cases.

# DURATION OF LABOR

Reference		No. of patients		Results	
1. Hingson and Edwards <sup>10</sup>	33	39	61	"Cervix seemed to dilate more rapidly"	
2. Block and Roehberg <sup>1</sup>	39	Average total labor in primipara, 3 hours;		Average time from injection to delivery in	
3. Block and Rotstein <sup>5</sup>	61	average total labor in multipara, 2 hours		primipara, 4 hours; in multipara, 2 hours	
4. Siever and Mousel <sup>15</sup>	300	"Over-all length of labor is increased"		Precaudal average, 8 hour; caudal average,	
5. Lyons and Hansen <sup>25</sup>	200	No comment		1-4 hours	
6. McCormick <sup>27</sup>	100	Labor shortened		Average first stage in primipara, 8 hours	
7. Lull <sup>19</sup>	553	1st		Average first stage in primipara, 8 hours	
8. McElroy and Donnelly <sup>28</sup>	162	Primipara 6:10	1:42	2nd	3rd
		Multipara 4:37	1:43		
9. Jarvis <sup>19</sup>	70	Primipara—average dilatation when in-		jection	
		jected 5.3 cm.; delivery 1:54 hours after		Average first stage 11:18, second stage	
		1:03, third stage: 09		Multipara—average amount of dilatation	
		on injection 4.6 cm.; average time to		delivery 1:54 hours	
		Average first stage 9:45, second stage: 27,		third stage: 10	

The wide variation in the duration of labor quoted above can be explained on the basis of different definitions of the length of labor. A shortened labor has been attributed to almost every new analgesic agent. Improved uterine motility and relaxation of the cervix would, perhaps, decrease the duration of labor. Unfortunately no objective evidence has been advanced to show that any analgesic agent has such an effect on the cervix. Uterine motility, on the other hand, has been registered objectively. Tocographic studies have shown that no essential change takes place in the rate or character of uterine contractions during serial caudal analgesia.<sup>12,30</sup>

**Summary.** Continuous or serial caudal analgesia is one of the most important advances in obstetrical analgesia since Simpson introduced the use of ether in 1847. The advantages of caudal analgesia during labor are so desirable that every effort should be made to eliminate any hazards inherent in the method.

It provides complete and dramatic pain relief in a large percentage of the patients when administered by experts. It relaxes the pelvic floor to a marked degree which is especially beneficial in cases of premature labor and when forceps are required. It is less depressing to fetal respiration than many of the other pain-relieving drugs. All of these features are highly desirable.

On the other hand, its use is attended by certain dangers. The first and foremost is the maternal risk. This has been emphasized by McCormick,<sup>27</sup> Block and Roehberg<sup>2</sup> and Studdiford commenting on Lull's<sup>23</sup> report. The administration of any depressant drug into the spinal canal carries with it a certain amount of risk. The chief maternal dangers of caudal analgesia are: (a) unrecognized subarachnoid tap, (b) the administration of massive doses of anesthetic drug, (c) infection in and about the spinal cord, (d) unexplained peripheral vascular collapse and, finally (e) sensitivity to the anesthetic solution.

Another disadvantage of caudal analgesia is the increased risk to the fetus. Although it is accompanied by less fetal respiratory depression, the rate of operative delivery is increased markedly. Simple operative procedures are easily carried out under caudal analgesia because of the marked pelvic floor relaxation which it provides. On the other hand, the increase in the number of posterior occiput positions, the number of infants in which the presenting part becomes arrested in the transverse position implies an increase in the number of more serious forceps applications.

The increased risk to the fetus and to the mother are important arguments against the use of caudal analgesia. However, they do not necessarily preclude a further study of this method with the hope that these dangers may be eliminated.

The obvious conclusion of these observations is that in spite of recent innovations in the field of obstetrical analgesia, the ideal is yet to be found.

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# PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF APRIL 18, 1944

**Prevention of Absorption and of Convulsions by Mercurial Diuretics.** R. BEUTNER, B. CALERNICK and P. BRADLOW (Dept. of Pharmacology, Hahnemann Medical Coll.). The convulsions from procaine, picrotoxin or strychnine are inhibited when salyrgan or mercurin are added to the solution of the convulsant prior to injection but not on separate injection. Addition of theophylline to the salyrgan or mercurin abolishes this inhibition. The absorption of a diffusible dye like phenol-red from the muscle into the blood stream is delayed when mercurin or salyrgan is added to the solution of that dye, but not on separate injection. If theophylline is also added there is no such delay of absorption. After intravenous injections, in rabbit, or after lymph sac injection, in frogs, the 3 convulsant drugs named will lead to convulsions even if salyrgan or mercurin is directly added. Diffusion experiments through parchment or rabbit's bladder show that there is no formation of a non-diffusible compound in this case since salyrgan or mercurin has no influence on the *in vitro* diffusion. The diffusion of phenol red through parchment is insignificantly accelerated by salyrgan and left unchanged by mercurin. Histologic studies indicate that salyrgan may have a protein coagulating effect *in vivo*. The antagonistic effect of theophylline, described above, seems to be due to a prevention of protein coagulation by the theophylline, as can be demonstrated in simple precipitation tests on serum proteins.

In conclusion: Mercurial diuretics inhibit absorption at the site of injection. Naturally they also inhibit their own absorption—the mercurial diuretic stays at the site of injection producing inflammation, so that some of it fails to reach the kidney. This can be avoided by addition of theophylline as is now invariably done in medical practice. It is unlikely that the described local effect of these mercurials is of importance for their diuretic effect.

**Ketone Body Formation from Fatty Acids, Using Heavy Carbon as a Tracer.** SIDNEY WEINHOUSE, GRACE MEDES and NORMAN FLOYD (The Lankenau Hospital Research Institute, Philadelphia). n-Octanoic acid containing 5.5% of the heavy carbon isotope,  $C^{13}$  in the carboxyl group, was incubated with liver slices from fasted rats. The resultant acetoacetic acid was broken down to acetone and  $CO_2$ , and the  $C^{13}$  content of each fraction determined. The excess  $C^{13}$  was found to be distributed equally between the acetone and carboxyl moieties. According to the classical theory of  $\beta$ -oxidation the ketone bodies arise from the terminal 4-carbons and hence should contain no excess isotope. According to the theory of multiple alternate oxidation, oxidation occurs at alternate carbons throughout the fatty acid chain prior to splitting into 4-carbon units; thus the resultant acetoacetic acid should contain the excess isotope exclusively in the carboxyl group.

The observed distribution of the carbon isotope in the acetoacetic acid provides unequivocal evidence for a mechanism of ketone body synthesis.

involving  $\beta$ -oxidation throughout the fatty acid chain, followed by a random coupling of the 2-carbon units. A similar experiment with carboxyl-labeled butyric acid indicates that the same mechanism is followed in the formation of acetoacetic acid from this fatty acid. Only a small portion of the isotopic octanoic acid which disappeared could be accounted for by the formation of ketone bodies or by complete oxidation to  $\text{CO}_2$ . The results of the experiments indicate that most of the octanoic acid utilized must have undergone a non-oxidative reaction leading to its fixation in the tissue.

**The Oxygen Metabolism of the Monkey's Brain in Vivo.** CARL F. SCHMIDT, HARRY H. PENNES and SEYMOUR S. KETY (Department of Pharmacology, Univ. of Penna.).\* In rhesus monkeys, lightly anesthetized with neubutal supplemented by pentothal as required, cerebral blood flow was measured by a method previously described (*J. Physiol.*, 138, 421, 1943) during the collection of samples of arterial and cerebral venous (jugular bulb) blood, which were analyzed for  $\text{O}_2$ ,  $\text{CO}_2$  and glucose content. The most important results were as follows:

1. *The magnitude of cerebral oxygen consumption under "normal" conditions.* In the 9 animals showing greatest signs of cerebral activity (corneal reflex active, spontaneous movements, vigorous respiration, good blood pressure) this averaged  $3.6 \pm \text{s.d. } 0.7 \text{ cc. O}_2 \text{ per } 100 \text{ gm. of brain (wet weight) per minute}$ , corresponding with a  $\text{QO}_2$  of 10.8; the cerebral arteriovenous oxygen differences averaged  $8.1 \pm \text{s.d. } 1.4 \text{ vols. \%}$ . The cerebral blood flows averaged  $45.6 \pm \text{s.d. } 12.4 \text{ cc. per } 100 \text{ gm. per minute}$ . The blood pressures varied from 84 to 135 and averaged 118 mm. Hg. The cerebral respiratory quotients averaged 0.97. The oxygen consumed by the brain averaged  $9.5 \pm \text{s.d. } 1.8 \%$  of the animal's total oxygen consumption.

2. *Variations in cerebral oxygen consumption under different circumstances.* The various observations can be grouped, according to the existing state of cerebral activity, as follows:

Group 1, in which respiration had ceased, vasomotor depression was present, and all reflexes had disappeared. Cerebral oxygen consumption in this group averaged  $1.66 \pm \text{s.d. } 0.8 \text{ cc. per } 100 \text{ gm. per minute}$ . Group 2, in which breathing was present though abnormally slow but no reflexes could be detected. Here cerebral  $\text{O}_2$  uptake averaged  $2.56 \pm \text{s.d. } 0.79 \text{ cc. per } 100 \text{ gm. per minute}$ . Group 3, with active circulation, respiration and reflexes. Cerebral oxygen consumption in this group averaged  $3.79 \pm \text{s.d. } 0.94 \text{ cc. per } 100 \text{ gm. per minute}$ . The differences among these 3 mean values are statistically significant.

Stimulant drugs (metrazol, picrotoxin) increased cerebral  $\text{O}_2$  consumption consistently when convulsions occurred, but had no effect otherwise. Pentothal decreased cerebral  $\text{O}_2$  consumption while depressing respiration and reflex activity.

Intentional decrease in cerebral blood flow by bleeding was associated with an increase in the cerebral arteriovenous oxygen difference. As long as the latter could nearly compensate for the former and prevent a considerable fall in the cerebral oxygen uptake, functional activity was retained, but once cerebral  $\text{O}_2$  uptake began to fall, reflexes were quickly lost and respiration became slower or ceased entirely. When the blood was reinfused, reflex activity was restored.

\* Work done under contract with the Office of Scientific Research and Development.

jected, some animals showed complete recovery and some did not; in the former, cerebral  $O_2$  consumption came back to or above the control level, while in the latter it did not. These findings confirm those previously made in dogs (*Am. J. Physiol.*, 84, 223, 1928).

3. *Correlations among cerebral metabolism, cerebral blood flow and cerebral A-V oxygen difference.* As between the A-V oxygen difference and cerebral blood flow (corrected for difference in blood pressure), there is no trace of significant correlation ( $r = 0.02$ ). Between A-V oxygen difference and cerebral metabolism a fairly significant correlation exists ( $r = 0.44$ ). The best correlation is between the cerebral metabolism and cerebral blood flow ( $r = 0.636$ ). This strongly suggests that there is an intrinsic mechanism whereby the tone of the cerebral blood-vessels is adjusted to the metabolic requirements of the brain. If so, cerebral A-V oxygen differences cannot be interpreted in terms of cerebral blood flow, for if this mechanism functions properly changes in the A-V oxygen difference will be prevented by vascular readjustments and any changes that occur will signify only that the adjustments were not completely adequate. Changes in the A-V difference tended to parallel cerebral metabolic changes, but not uniformly; the A-V oxygen difference was increased in some experiments by both metrazol (or picrotoxin) and pentothal although cerebral metabolism was increased by the former and decreased by the latter.

4. *Relation of these to other figures for cerebral metabolism.* The  $QO_2$  in our best experiments ranged from 7.5 to 13.5 and averaged 10.8. These figures are much smaller than any previously reported for the metabolism of the brain *in vivo* (*Am. J. Physiol.*, 140, 190, 1943), the  $QO_2$  values ranging from about 17 to 39. Since ours are the only experiments in which reasonably accurate blood flow measurements have been made we attribute the higher figures reported by others to overvaluation of the blood flow factor, because of escape of blood to extracranial areas or other technical defects. Figures for the  $QO_2$  of cortical slices or homogenates *in vitro* range from 10.7 to 15.5, which is about the order of our highest values. At present we do not know whether this agreement is fortuitous or significant because we do not know how much of the metabolism and blood flow measured in our experiments represents cortical activity, or how much the anesthetic might be expected to depress the  $QO_2$  in our experiments.

Observations on the Anticephalin Activity of Normal and Hemophilic Plasmas. L. M. TOCANTINS (Division of Hematology, Jefferson Medical Coll., Philadelphia). Exposure of plasma to adsorbents (asbestos fibers, kaolin, infusorial earth, pumice stone) reduces its anticephalin activity. Contact of 1 cc. of plasma for 60 minutes at  $20^\circ C$ . with 50 mg. or more of asbestos fibers removes most of the prothrombin and some of the fibrinogen from the plasma. By adjusting the conditions of the contact exposure, it is possible to remove most of the anticephalin, without greatly disturbing the other clotting factors. Such "adsorbed plasmas," whether normal or hemophilic, behave alike towards cephalin, clot spontaneously, though slowly, even in the presence of citrate, and at approximately the same rate whether in glass or paraffin coated tubes. The rate of conversion of hemophilic prothrombin into thrombin by cephalin is slower than normal. Dilution of the plasma (1-20, 1-30) equalizes the rate of conversion and nearly effaces the difference in behavior towards cephalin between normal

and hemophilic plasmas. The prothrombin conversion rate of "adsorbed plasma" is faster than normal. Higher dilutions (1-60, 1-80) are necessary to equalize the prothrombin conversion rate of normal plasma with that of "adsorbed plasma." Prothrombin estimations done on "adsorbed plasma" by the 1-stage method (Quick) yield higher values than by the 2-stage method (Smith). The increase in the prothrombin conversion rate which seems to follow removal of antiecephalin from the plasma may explain this disagreement between the results obtained by the 2 methods.

**Depressor Effect of Cold Upon the Static Receptors of the Labyrinth.** E. A. SREGEI (Dept. of Exp. Neurology, Temple Univ. School of Medicine, Philadelphia). A u-shaped cannula introduced into the external and middle ear of decerebrate cats was perfused with water of from 3° to 20° C. Water of 9° or below produced within a few minutes a decrease in the muscle tone of the homolateral foreleg, causing a drooping of this leg. This effect may outlast the caloricization, but is reversible. Similar, although less pronounced, effects were observed in rabbits with intact brain. The effect of unilateral cooling upon the posture of the head was demonstrated in unanesthetized rabbits and in cats under bulbocapnine catalepsy. The cold temporarily produced effects similar to those of unilateral labyrinth paralysis: a rotation of the head about the oro-occipital axis, the affected ear lying lower. The excitability of the cristae revealed no significant difference of the postrotatory nystagmus in cats rotated before and during prolonged application of cold to both ears. Thus a depressor effect of cold could be observed only on static receptors influencing the posture of head and extremities (apparently chiefly the maculae), while such an effect upon the receptors reacting to angular acceleration was not noticeable. The possible role of physical factors in disorientation (change in specific gravity of the endolymph altering the otolithic pressure upon the macula).

# BOOK REVIEWS AND NOTICES

CLINICAL DIAGNOSIS BY LABORATORY EXAMINATIONS. By JOHN A. KOLMER, M.S., M.D., Dr.P.H., Sc.D., LL.D., L.H.D., F.A.C.P., Professor of Medicine in the School of Medicine and the School of Dentistry of Temple Univ.; Director of the Research Institute of Cutaneous Medicine; Formerly Professor of Pathology and Bacteriology in the Graduate School of Medicine of the Univ. of Penn. Pp. 1239; 75 figs. New York, London: D. Appleton-Century, 1943. Price, \$8.00.

This new work, by a well-known writer of successful articles and textbooks, is presumably an outgrowth from his earlier books, "Infection, Immunity, and Specific Therapy" and "Approved Laboratory Technique" (with Boerner), with which there is necessarily considerable overlap. In its Part 2, "The Practical Applications of Laboratory Examinations in Clinical Diagnosis," however, it cultivates new ground. Here, the expected laboratory findings are given for a number of important diseases. For instance, in the chapter on Diseases of the Blood and Hemopoietic System (57 pages), some 40 odd diseases are considered. The high points of each are presented in one or more opening paragraphs, then the laboratory tests to be used and the results to be expected. This will undoubtedly be a useful and convenient form of presentation for many clinicians who do not feel up to date in their knowledge of the application of laboratory tests to clinical medicine. It should hardly be necessary to point out that the picture of the disease itself is of course far from adequate, nor is it meant to be. Yet the slothful might be tempted to make it substitute for the more complete treatment given in the conventional textbooks of medicine.

Part I, the larger half of the book, covers "the clinical interpretation of laboratory exams." Here, brief statements about the normal condition of the material in question are followed by descriptions of the various changes observed—often in convenient tabular form—how they are brought about, and their significance. There is also a shorter section on the technique of laboratory exams. Thus, the book contains material of value to clinicians, laboratory diagnosticians and technicians, and teachers and students of "clinical pathology." One is chiefly struck by the amount of information offered—the Table of Contents covers 32 pages, for example, and the Index 130 pages. One wonders that a single individual—and a busy one at that—could have collected such a mass of information covering so many fields; and one should not be surprised that the book has more than the average number of typographic errors and statements which many will find incorrect or open to question. Relatively, these are few, however, and more than outweighed by the book's good points.

THE HIPPOCRATIC OATH. Text, Translation and Interpretation by LUDWIG EDELSTEIN. Supplements to the Bull. of Hist. of Med., Editor: HENRY E. SIGERIST, No. 1. Pp. 64. Baltimore: Johns Hopkins Press. Price, \$1.25. This is the first of a series of Monographs, published occasionally as supplements to the *Bulletin of the History of Medicine*. The series which has been in prospect for some years aims to accommodate articles too long for the *Bulletin*, but not long enough to be published as independent monographs. The supplements are sold independently and at reduced prices to those already receiving the *Bulletin*. We congratulate the Institute on the achievement of this new series.



Dr. Edelstein, a recognized authority on Greek medicine, presents a detailed and well-documented interpretation of the famous Oath, an interpretation derived chiefly from the regulations about poisons and abortions. It is regarded as a Pythagorean document, composed not before or after the 4th century, *i. e.*, like so much of the Corpus Hippocraticum, not the work of the great Hippocrates himself. The Author states positively that the Oath was not at first accepted by all, but that it began to be popular at the end of antiquity, eventually to become "the nucleus of all medical ethics."

E. K.

THE RELIGIOUS AND PHILOSOPHICAL ASPECTS OF VAN HELMONT'S SCIENCE AND MEDICINE. By WALTER PAGE. Supplements to the *Bull. of Hist. of Med.*, No. 2, Editor: HENRY E. SIEGIST. Pp. 44. Baltimore: Johns Hopkins Press. Price, \$1.00.

VAN HELMONT'S contributions to medical progress as an iatrochemist and explainer of vital phenomena in chemical terms and his invention of the word and concept "gas" are well known. His contributions to philosophy, however, are, in the opinion of the Author, poorly comprehended and appreciated. This he aims to rectify, integrating van Helmont's religio-philosophic with his scientific side, in order to present a true picture of the whole of van Helmont's study of life.

E. K.

A SURGEON'S WORLD. An Autobiography. By MAX THOREK, M.D. Pp. 410. Philadelphia, New York: J. B. Lippincott, 1943. Price, \$3.75.

"A Surgeon's World" is the full-length autobiography of the eminent, Hungarian-born, American surgeon, Max Thorek. It is an intimate account of his life from his earliest remembrances as a boy in his native town among the Tatra Mountains, as a student in Budapest, and as an immigrant citizen in the land of great opportunity—America! The story is excellently written, and as he relates the experiences and problems that confronted him during his life as a member of the medical profession, one cannot help but "live" along with the Author.

Throughout the entire story, the reader appreciates and observes the philosophy of Dr. Thorek's life which is unique among those who were born and reared on "the battleground of Europe," and who subsequently came to America where freedom and opportunity are the heritage of everyone.

The book is filled with many interesting anecdotes of both a professional and a humanitarian nature that are constantly confronting a surgeon. At the end of the book, Dr. Thorek gives a short résumé of the outstanding pioneers in the field of medical science from the time of Hammurabi to the present. This list is conspicuous, however, by the absence of the immortal names of Marie and Pierre Curie, and one questions the wisdom of trying to cover such a big topic in this way and in a book of this kind.

This book is a doctor's book which tells of Dr. Thorek's work, those who helped him as well as those who were helped by him. It is forthright, impartial and unaffected. It reflects a fine gusto for life and activity and a point of view that is as aware of the humorous as of the pitiable. It is an entertaining book and the many people who are destined to read it shall consider the time probably spent.

WHAT IS HYPNOSIS: Studies in Auto and Hetero Conditioning. By ANDREW SALTER. Pp. 88. New York: Richard R. Smith, 1944. Price, \$2.00.

"HYPNOSIS is the production of reactions in the human organism through the use of verbal or other associative reflexes." Continuing, the writer proceeds to show that it is but a conditioned reflex. Thus is "suggestion" thrown to the discard and Pavlov's "conditioned response" assigned to its place. The chapters are: What is hypnosis? a further consideration of

hypnosis; preliminary experiments in autohypnosis; a study of Group III; appendix: three techniques of autohypnosis.

"Rapport" is considered an obsolete word; "hypnotist" is an unfortunate term; and a more appropriate designation for "conditioned reflex" it is said would be "associative reflex." Previously, it had been believed that through hypnosis persons could not be impelled to commit crimes; but now, "hypnotized subjects have stolen money, rushed to pick up rattlesnakes, and thrown sulphuric acid into a man's face, which, unknown to the subject was protected by invisible glass." That such a menacing agent may not be released in our midst seems assured, however, since it is said, "I doubt that at present more than half a dozen persons in this country could command technique sufficient to do so."

Finding an appropriate answer to this interrogatory title is admittedly difficult; nevertheless, the Author believes it has been achieved. A weakness of the theme is a tendency to employ chiefly those references that support the Author's views.

**HUMAN CONSTITUTION IN CLINICAL MEDICINE.** By GEORGE DRAPER, M.D., Associate Professor of Clinical Medicine, Coll. of Physicians and Surgeons, Columbia Univ.; Associate Attending Physician, Presbyterian Hospital, New York City; C. W. DUFERTUIS, Ph.D., Physical Anthropologist, Constitution Clinic, Presbyterian Hospital, New York City; and J. L. CAGNEY, JR., M.D., M.P.S.C.D., Associate in Medicine, Coll. of Physicians and Surgeons, Columbia Univ.; Assistant Physician, Presbyterian Hospital, New York City. Pp. 273; 28 figs.; 30 tables. New York, London: Paul B. Hoeber, Med. Dept. of Harper & Brothers, 1944. Price, \$4.00.

This book represents an attempt to define the man within the patient. In the end failure is admitted, since the spirit of man is imponderable. At the same time, on the basis of morphology, physiology, immunity and emotion, a good case is made out for the primary thesis: that disease depends not merely on the nature of the external injurious agent and the usual disease process but equally on the nature of the total personality of the particular person affected. This leads the Authors into a detailed description and discussion of genetics, androgyny (the composite of masculine and feminine characteristics), anthropometry, somatotypes, constitutional physiology and psychology. They wisely point out the importance of a broad cultural background for the practice of medicine and show how the man within the patient, instead of obstructing the healer's efforts, may, if properly understood, become his effective ally. The book should be read by every student of medicine in his effort to develop a good "bedside manner," which the Authors define as "a distillate of objectivity, intuition, common sense and good manners." More important, however, is this successful adaptation to clinical practice of the known facts in the study of the constitution, a necessarily detailed and protracted field of study in which the senior author has been outstanding for many years.

**THE SEXUAL GLANDS OF THE MALE.** By OSWALD SWINNEY LOWSLEY, A.B., M.D., F.A.C.S., Collaborators: FRANK HINMAN, A.B., M.D., F.A.C.S., DONALD R. SMITH, A.B., M.D., and ROBERT GUTIERREZ, A.B., M.D., F.A.C.S., Reprinted from Oxford Loose-Leaf Urology. Pp. 619; 4 tables; 59 figs. New York: Oxford Univ. Press. Price, \$10.00.

This book, written by well-known authors in the urologic field, is composed of material reprinted from the "Oxford Loose-Leaf Urology." Because knowledge concerning the sexual glands of the male has accumulated so rapidly in the past decade, it is of considerable value to have this information in a single volume. Much of the subject matter recorded is from the original works of the authors. However, there is extensive citation of the literature. Bibliographies follow each section of the book.

Dr. Lowley writes the section on the prostate gland; Drs. Hinnman and Smith, the section on the testicle and epididymus; and Dr. Gutierrez, the section on the seminal tract and spermatic cord. Illustrations are by Mr. Wil-

liam P. Didusch.

In each of the sections the embryology, anatomy, physiology and pathology of the particular organ being discussed are presented. The latest methods of surgical and non-surgical therapy are discussed as each organ is presented. The author's personal methods of treatment are emphasized, but opinions of others recorded in the literature are given on controversial subjects. The book appears to be written primarily for those interested in a specialized knowledge of the genital tract of the male. It should prove valuable as an authoritative reference to the urologist, general surgeon and endocrinologist.

L. LAY.

**AUTHORITY IN MEDICINE: OLD AND NEW.** By MAJOR GREENWOOD, D.Sc., F.R.C.P., F.R.S., Professor of Epidemiology and Vital Statistics in the Univ. of London. The Linaere Lecture, May 6, 1943. Pp. 32. Cambridge: Univ. Press; London: Bentley House; New York: Macmillan, 1943. Price, \$40.

We are glad to commend this essay to our readers. Pertinently for a Linaere Lecture, it considers Galen, the authoritative—with a kind word for his interpreter, Linaere—but it is Galen in the true original, not in the perverted and shrivelled authoritarianism that has been handed us by the middle Ages. Galen on personal hygiene, dietetics, physical training, and as a medical psychologist usually gave sound practical advice, though his reasons for it often were fallacious. At any rate, he was by no means the dogmatist that we have been led to believe by his commentators of 1000 years.

Major Greenwood, a thorough believer in the experimental method, nevertheless recognizes its limitations and apparently agrees with Raymond Pearl that "of all methodological procedures in biology, the *experimentum crucis* is the most dangerous," and that many such, loudly hailed, have later proved to have led to false conclusions. Yet, he foresees a future for experimental epidemiology, especially on statistical lines. The essay concludes with a consideration of "the authority of intention," upholding the zealous maintenance of pure research and intellectual freedom.

E. K.

**THE RIDDLE OF CANCER.** By CHARLES OBERLING, M.D. Translated by WILLIAM H. WOGLOM, M.D. Pp. 196. New Haven: Yale Univ. Press; London: Oxford Univ. Press, 1944. Price, \$3.00.

PROFESSOR OBERLING is a distinguished student of cancer, well qualified from personal experience to write upon this subject. In addition, his unusual ability to write and to teach him peculiarly fitted to present the subject of cancer in a readable and understandable manner. Dr. Woglom has done an excellent job of translating. This little volume traces the development of our concepts of cancer from the earliest available records to the most modern hypotheses, concisely and clearly and with a commendable absence of irrelevant material. Three hypotheses are presented, the vitational, the embry-mental cancer. Transplantable tumors are considered in some detail, as are also induced tumors. Of the latter, the greatest emphasis is laid upon the carcinogenic viruses; Professor Oberling's main theme throughout the book is an enthusiastic support of the virus hypothesis as the cause of cancers in general. As he states it, "Either we must agree that there are many different kinds of malignant growth, and thus be free to admit as many causes as we wish; or that there is but one malignant process, in which case the virus hypothesis becomes the only logical solution." In answer to those who may be inclined to criticize the author for his whole-hearted acceptance of the virus hypothesis, his closing sentence is addressed: "To those who may be inclined

to reproach the author for a too enthusiastic partisanship he would reply that it is never ill judged to be guided by a hypotheses so long as it does no violence to the known facts, and that the best proof of value is the amount of research stimulated. Submitted to these tests, the virus hypothesis has nothing to fear."

To the Reviewer the greatest value of the book lies in the separation of the grain from the chaff in the data obtained upon cancer by research. The significant material is included and carefully interpreted. The layman may meet with difficulty in understanding some of the terminology and some of the reasoning may also leave him confused upon the first reading; but from it all he should be able to grasp the essentials. For the medical student, physician, and even for the cancer investigator, the book holds a wealth of material in its relatively few pages. The Reviewer enthusiastically recommends it to everyone interested in our present-day concepts of cancer.

A TEXT-BOOK OF PATHOLOGY. Edited by E. T. BELT, M.D. Contributors: E. T. BELT, M.D., Professor of Pathology, Univ. of Minnesota; B. J. CLAWSON, M.D., Professor of Pathology, Univ. of Minnesota; and M. S. MCCARTNEY, M.D., Associate Professor of Pathology, Univ. of Minnesota. Fifth ed. Pp. 862; 448 figs. (4 color plates). Philadelphia: Lea & Febiger, 1944. Price, \$9.50.

The 5th edition of this text represents a thorough revision and the introduction of much new material, yet with a reduction of 69 pages. Of particular interest is the inclusion of recent work on blast injuries and infectious diseases of interest in war medicine. B. J. Clawson has contributed the chapter on Diseases of the Heart, and J. S. McCartney, the chapters on The Mycoses, and on Diseases of the Liver and Gall Bladder. Even in a single volume text-book of this size, there are omissions of material that, to the Reviewer, should be included. Also, the quality of the illustrations is occasionally unsatisfactory, and one misses a statement of magnifications and stains in the legends. Useful bibliographies are appended at the end of each chapter, though, in not a few instances, they are not sufficiently up to date. This book, however, appeals to the Reviewer as a generally sound and readable text and should fulfill the Authors' desire to furnish medical students with a text-book which may be of use during the clinical years as well as during their course in pathology.

## NEW BOOKS

*Anatomy and Physiology. Laboratory Manual.* By CATHERINE PARKER ANTHONY, B.A., R.N., Instructor of Anatomy and Physiology, Lutheran Hospital; Formerly Instructor of Anatomy and Physiology, St. Luke's Hospital, and Assistant Instructor of Anatomy and Physiology, Frances Payne Bolton School of Nursing, Western Reserve Univ., Cleveland, Ohio. Pp. 249; many illus. St. Louis: C. V. Mosby, 1944. Price, \$2.00.

*The Jews and Medicine Essays.* By HARRY FRIEDENWALD, M.D., D.H.L. (Hon.), D.Sc. (Hon.), Professor Emeritus of Ophthalmology, Univ. of Maryland. Preface by HENRY E. SIEGERT. Publications of the Institute of the History of Medicine, The Johns Hopkins Univ. First Series: Monographs. Vols. II and III. Pp. 817; many figs. Baltimore: Johns Hopkins Press, 1944. Price, \$3.75 per vol.

*Allergy in Practice.* By SAMUEL M. FEINBERG, M.D., Associate Professor of Medicine and Chief of Division of Allergy, Northwestern Univ. Medical School; President, American Association for the Study of Allergy, 1942-43. With the Collaboration of OREY C. DURHAM, Chief Botanist, Abbott Laboratories. Pp. 798; 36 figs. Chicago: Year Book Publishers, 1944. Price, \$8.00.

*The Wounded Get Back.* By ALBERT Q. MAISEL. Foreword by ROSS T. McINTIRE, Rear Admiral, M.C., U.S.N., The Surgeon-General of the Navy. Pp. 280. New York: Harcourt, Brace, 1944. Price, \$2.50.

*The Brush Foundation Study of Child Growth and Development.* I. Psychometric Tests. By ELIZABETH EBERT and KATHERINE SIMMONS. Monographs of the Society for Research in Child Development, Vol. VIII, No. 2, Serial No. 35. Pp. 113; 19 figs.; 70 tables. Washington, D. C.: National Research Council, 1943. Price, \$1.50.

*Small Community Hospitals.* By HENRY J. SOUTHWALL, Director, Division of Rural Hospitals, The Commonwealth Fund, and GEDDES SMITH, Associate Director, The Commonwealth Fund. Foreword by BARRY C. SMITH, General Secretary, The Commonwealth Fund. Pp. 182. New York: The Commonwealth Fund, 1944. Price, \$2.00.

*The War and Mental Health in England.* By JAMES M. MACKINTOSH, M.D., Professor of Preventive Medicine, University of Glasgow. Pp. 91. New York: The Commonwealth Fund, 1944. Price, \$3.50.

*Synopsis of Neuropsychiatry.* By LOWELL S. SELLING, Sc.M., M.D., Ph.D., Dr. P. H., Director, Psychopathic Clinic, Recorder's Court, Detroit Mich.; Associate Attending Neuropsychiatrist, Eloise Hospital; Adjunct Attending Neuropsychiatrist, Harper Hospital. Pp. 500; numerous figs. and tables. St. Louis: C. V. Mosby, 1944. Price, \$5.00.

*Virus Diseases in Man, Animal and Plant.* A Survey and Reports Covering the Major Research Work Done During the Last Decade. By GUSTAV SEIFFERT. Translation by MARION LEE TAYLOR, Ph.D. Pp. 332; 7 figs.; 7 tables. Published upon recommendation of the National Research Council. New York: Philosophical Library, 1944. Price, \$5.00.

## NEW EDITIONS

*Clinical Urology.* By OSWALD SWINNEY LOWMEYER, A.B., M.D., F.A.C.S., Director of Dept. of Urology (James Buchanan Brady Foundation) of the New York Hospital; and THOMAS KIRWIN, M.A., M.S., M.D., F.A.C.S., Attending Surgeon of Dept. of Urology (James Buchanan Brady Foundation) of the New York Hospital. Drawings by WILLIAM P. DIPUSSEN. Second ed. Vols. 1 and 2. Pp. 1884; 220 figs. Baltimore: Williams & Wilkins, 1944. Price, \$10 set of 2 Vols.

*Textbook of Gynecology.* By EARL NOVAK, M.D., F.A.C.S., Associate in Gynecology, The Johns Hopkins Medical School; Gynecologist, Bon Secours and St. Agnes Hospitals, Baltimore. Second ed. Pp. 708; 456 figs. Baltimore: Williams & Wilkins, 1944. Price, \$8.00.

*Manual of Urology.* By R. M. LECOMTE, M.D., I.A.C.S., Professor of Urology, Georgetown Univ. Medical Department; Member of the American Urological Association. Third ed. Pp. 305; 60 figs. Baltimore: Williams & Wilkins, 1944. Price, \$4.00.

*The Art and Science of Nutrition.* A Textbook on the Theory and Application of Nutrition. By ESTELLE H. HAWLEY, Ph.D., and GRACE GARDEN, B.S., of Rochester Municipal Hospitals, Rochester, N. Y. Second ed. Pp. 668; 139 illus (11 colored); 138 figs.; 68 tables. St. Louis: C. V. Mosby, 1944. Price, \$3.75.

*The Principles and Practice of Medicine.* Originally written by Sir WILLIAM OSLER, BARTO, M.D., F.R.C.P., F.R.S. Designed for the use of practitioners and students of medicine by HENRY A. CHRISTIAN, A.M., M.D., LL.D. (Hon.), Sc.D., Hon. F.R.C.P. (Can.), F.A.C.P. Fifteenth ed. Pp. 1498. New York: D. Appleton-Century, 1944. Price, \$9.50.

*Elimination Diets and the Patient's Allergies.* A Handbook of Allergy. By ALBERT H. ROWE, M.D., Lecturer in Medicine, Univ. of California Medical School, San Francisco, Calif.; Consultant in Allergic Diseases, Alameda County Hospital, Oakland, Calif. Second ed. Pp. 256. Philadelphia: Lea & Febiger, 1944. Price, \$3.50.

*Tropical Nursing.* A Handbook for Nurses and Others Going Abroad. By A. L. GREGG, M.A., M.D., M.Ch., B.A.O. (Dublin), D.T.M., and H. (LOND.), L.M. (ROTUNDA HOSPITAL); Member of Associate Staff of, and Lecturer to Nurses at the Hospital for Tropical Diseases, London. Lecturer on Tropical Diseases, Westminster Hospital Medical School. Second ed. Pp. 185, 13 figs. New York: Philosophical Library, 1944. Price, \$3.00.

*Synopsis of Diseases of the Heart and Arteries.* By GEORGE R. HERRMANN, M.S., M.D., Ph.D., F.A.C.P., Professor of Medicine, University of Texas; Director of the Cardiovascular Service, John Sealy Hospital; Consultant in Vascular Diseases, U. S. Marine Hospital. Third ed. Pp. 516, 103 figs.; numerous tables; 103 illus. (4 color plates). St. Louis: C. V. Mosby, 1944. Price, \$5.00.

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We hope that any unpleasant effect produced by cutting down the margins will be accepted and approved by readers as a temporary war casualty. It is possible that more radical changes will have to be made later, but we are loath to change any more than absolutely necessary, a format that has existed practically unchanged since the Journal began in 1820.

For the balance of the war, 150 reprints will be supplied gratis. Covers will be omitted on all articles. In ordering additional reprints, we will supply in multiples of 150. This modification is for the same reason as the change of format, i. e., conservation of paper.

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We desire to secure several copies of the March and May 1943 numbers of this Journal, in order to comply with requests and need for replacements in long library "runs." The war situation has made it impossible to print extra numbers to supply this demand. We would be very grateful to anyone who would return the Publishers any unutilized copies of these numbers for which they have no further use, and we would be glad to repay postage.







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